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# EFFICACY OF ADRENAL CORTICAL EXTRACT AND OF PAREDRINE IN THE PREVENTION OF EXPERIMENTAL SHOCK FOLLOWING VENOUS OCCLUSION OF A LIMB[1]

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Received for publication July 20, 1942

Recently, a method was developed in this laboratory for the experimental production of shock by occlusion of the veins in the hind leg of the dog (1). It was found that this procedure led to a state of shock followed by death in from 3½ to 21 hours in 87 per cent of the animals. In previous reports the prophylactic and therapeutic value of two procedures were discussed: namely, the use of desoxycorticosterone acetate (DCA) (2) and the application of a cast (3). In this report we wish to present our results with adrenal cortical extract and with paredrine.

ADRENAL CORTICAL EXTRACT. Several reports have appeared suggesting that adrenal cortical extract (ACE) is beneficial in preventing shock. Perla (4) found that cortin prevented the occurrence of histamine shock in adrenalectomized rats, and Heuer and Andrus (5) found that it had a beneficial effect on blood pressure and plasma-fluid loss in dogs following the intravenous injection of extracts from obstructed bowel loops. Selye et al. (6) found cortin effective in ameliorating shock in rats following intestinal trauma. Swingle et al. (7) prevented traumatic shock in dogs by the use of adrenal cortical extracts. We therefore thought it advisable to test this extract on the type of shock which follows occlusion of the veins of the limb.

*Method.* As previously described, the common and internal iliac veins of one limb were ligated aseptically using a retro-peritoneal approach. This was followed by the injection of 10 to 15 cc. of a 1:20 suspension of sterile lampblack in normal saline (1). Measurements of blood pressure, heart rate, hematocrit and limb circumference were recorded at regular intervals following the operation. The dosage of adrenal cortical extract (ACE)[2] varied from 17.5 to 39.0 cc. subcutaneously—the average being 23.3 cc. (table 1). One-half of this amount was given 12 hours before the operation, and the remainder in divided doses during the first 12 post-operative hours.

*Results.* The results are summarized in table 1. Twelve dogs were used in this series, all but one having been given ACE preoperatively. Five animals

[1] Aided by the A. D. Nast and Emil and Fanny Wedeles Funds for Cardiovascular Research.

[2] We are grateful to Dr. D. Klein of Wilson Laboratories for generous supplies of ACE.

died within the first 24 hours after operation and necropsy indicated death due to shock. One dog died of confluent bronchopneumonia 24 hours after operation and did not appear to have developed shock. The remaining 6 animals survived.

The five animals dying of shock were found to have developed a maximum limb enlargement up to 34 per cent beyond the control size, with an increase in

TABLE 1

*Effect of adrenal cortical extract (ACE) on the shock-like state following venous occlusion of the hind limb of the dog*

| DOG NO. | Weight | ACE, CC. SUBCUTANEOUSLY | | TOTAL | SURVIVAL TIME | PER CENT INCREASE OF THIGH CIRCUMFERENCE OVER CONTROL SIZE | HEMATOCRIT | BLOOD PRESSURE | INCREASE IN WEIGHT OF LEG WITH OCCLUDED VEIN IN PER CENT OF BODY WEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | Preoperative | Postoperative | | | | | | |
| | *kgm.* | | | | *hrs.* | | | | |
| 1 | 10.9 | 0 | 18 | 18 | 9 | 22 | No change | Dropped | 8.0 |
| 2 | 9.5 | 20 | 10 | 30 | 24* | 9 | Increased | Dropped | 1.8 |
| 3 | 17.2 | 10 | 10 | 20 | S† | 15 | Increased temporarily | Dropped temporarily | |
| 4 | 8.6 | 10 | 7½ | 17½ | 7 | 22 | Increased | Dropped | 6.2 |
| 5 | 10.4 | 10 | 10 | 20 | S | 6 | No change | Dropped temporarily | |
| 6 | 11.3 | 10 | 10 | 20 | S | 14 | No change | Dropped temporarily | |
| 7 | 17.7 | 10 | 10 | 20 | 12–22 | 20 | Slightly increased | Dropped | 3.0 |
| 8 | 10.4 | 10 | 10 | 20 | S | 28 | After 24 hrs. decreased | No change | |
| 9 | 16.3 | 10 | 12½ | 22½ | 12 | 34 | Increased | Dropped | 5.5 |
| 10 | 11.3 | 10 | 10 | 20 | S | 8 | Slightly increased then, after 24 hours, decreased | No change | |
| 11 | 7.7 | 20 | 19 | 39 | S | 26 | Slightly increased then, after 24 hours, decreased | No change | |
| 12 | 10.4 | 20 | 12½ | 32½ | 6 | 23 | Increased | Dropped | 5.5 |

* Died of bronchopneumonia.

† Survived the operation.

weight of the edematous limb from 3.0 to 8.0 per cent of the body weight. This represents a loss of plasma fluid into the limb equivalent to from 50 to 75 per cent of the total blood volume. Survival time of these animals was no longer than that of untreated dogs. All showed a drop in blood pressure and in all but one there was a definite hemoconcentration (table 1).

Among the six dogs that survived, limb enlargement did not progress beyond 28 per cent of the control size, but this occurred after a longer interval than

in those that succumbed. The blood pressure in these animals showed a definite transitory fall in all but 3; in these exceptions no change occurred. The hematocrit showed a slight increase in 3 dogs in the first 24 hours and no change in 3. Later hemodilution occurred in three animals (table 1).

*Discussion.* Our results are in accord with previous work indicating that ACE exerts a beneficial effect in preventing the occurrence of the irreversible development of shock. In our ACE series of dogs, 50 per cent of the animals survived, as compared with a survival of 13 per cent of the animals in the untreated control series (1 and 2). Among the six ACE treated dogs that survived, none showed signs of developing shock.

The percentage of survivals in the ACE series was not as great as in the DCA-treated series of dogs (2). This fact may indicate that DCA is a more effective therapeutic agent than ACE, or it may be merely a question of proper dosage. The ACE contains a large number of the various steroids, in undetermined amounts, present in the adrenal cortex. Until these substances are available

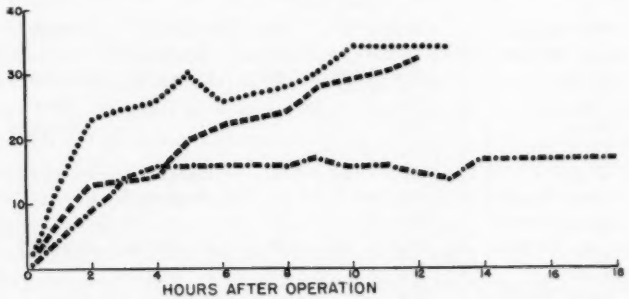


Fig. 1. This graph shows the average per cent increase in leg circumference over the control circumference following venous occlusion of the hind limb. Control series (2), dot curve. DCA series (2), dash curve. ACE series, dot-dash curve. Discussed in text.

in isolated form in sufficient amounts to permit their use in experiments such as ours, nothing can be said of their relative potency in preventing this form of shock.

The beneficial action of ACE may be either the prevention or diminution of local fluid loss into the occluded limb, or some action on the secondary mechanisms which are consequent to this decrease in circulating blood volume. In figure 1 is shown the average rate of leg enlargement of the control and primed DCA series previously reported (2), and the average rate of leg enlargement in the ACE series of the present study. This comparison shows clearly that ACE has a greater inhibiting effect on leg enlargement than DCA. There is thus no doubt that ACE diminishes local fluid loss. This action may be due in part to a decrease in capillary permeability, which has been shown to occur with ACE (8), or to some action on the osmotically active constituents of the blood, such as was indicated with DCA in our previous study (2).

Although ACE definitely inhibits local fluid loss, there is no evidence that it

may not also act on the secondary effects of decreased circulating blood volume. One possibility lies in the ability of ACE to conserve body sodium (9).

PAREDRINE. Paredrine, parahydroxyphenylisopropylamine, is a sympathomimetic drug which is more stable than epinephrine and almost entirely devoid of action on the central nervous system. Since it has a vasoconstrictor effect (10) it has been suggested as a therapeutic agent in shock to cause a sustained rise in the blood pressure (11), and thereby help maintain the flow of blood in

TABLE 2

*Effect of paredrine on the shock-like state following venous occlusion of the hind limb of the dog*

| DOG NO. | WEIGHT | PAREDRINE ADMINISTERED | | | SURVIVAL TIME | PER CENT INCREASE OF THIGH CIRCUMFERENCE OVER CONTROL SIZE | HEMATOCRIT | BLOOD PRESSURE | INCREASE IN WEIGHT OF LEG WITH OCCLUDED VEIN IN PER CENT OF BODY WEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | Time after operation | Amount | Route | | | | | |
| | *kgm.* | | *cc*.* | | *hrs.* | | | | *kgm.* |
| 1 | 11.8 | 1 hr. 15 min. | 1 | SQ† | 3 | 23 | Markedly increased | Dropped | 3.0 |
| | | 2 hr. 20 min. | 1 | IV† | | | | | |
| 2 | 10.9 | 35 min. | 1 | SQ | 8¼ | 20 | Increased | Rose and then dropped | 4.1 |
| | | 2 hrs. | 1 | SQ | | | | | |
| | | 4hrs. | 1 | SQ | | | | | |
| | | 7 hrs. 45min. | 1 | SQ | | | | | |
| 3 | 8.7 | 3 hrs. | 1 | IV | 6½ | 12 | Markedly increased | Dropped | 6.1 |
| | | 3 hrs. 30 min. | 1 | SQ | | | | | |
| | | 5 hrs. 15 min. | | IV | | | | | |
| 4 | 11.5 | 1 hr. 50 min. | 1 | SQ | 9¼ | 26 | No change | Dropped | 5.0 |
| | | 4 hrs. | 1 | SQ | | | | | |
| | | 5 hrs. 45 min. | 1 | SQ | | | | | |
| | | 6 hrs. 45 min. | 1 | SQ | | | | | |
| | | 7 hrs. 45 min. | 1 | SQ | | | | | |
| | | 8 hrs. 45 min. | 1 | SQ | | | | | |
| 5 | 11.0 | 1 hr. 15 min. | 1 | SQ | 11¼ | 30 | Markedly increased | Rose and then dropped | 5.2 |
| | | 3 hrs. 15 min. | ½ | SQ | | | | | |
| | | 7 hrs. 15 min. | 1 | SQ | | | | | |
| | | 9 hrs. 15 min. | 1 | SQ | | | | | |
| 6 | 9.0 | 45 min. | 1 | SQ | 5 | 7 | Markedly increased | Rose and then dropped | 3.8 |
| | | 2 hrs. 45 min. | ½ | SQ | | | | | |

* Each cubic centimeter is equal to 20 mgm. paredrine.

† SQ = subcutaneously; IV = intravenously.

the coronary vessels and other vital organs. Consequently, we tested paredrine on dogs in which shock was produced as previously described (1).

*Method.* The operative procedure was the same as that described above. Paredrine hydrobromide[3] was administered both subcutaneously and intravenously in doses varying from 30 to 120 mgm. per animal (table 2). The first dose was given immediately after the blood pressure began to fall, and was

[3] Generously supplied by Smith, Kline and French Laboratories.

repeated as soon as a further blood pressure drop was noted. Similar observations on heart rate, hematocrit and limb measurements were taken as in the group with ACE.

*Results.* In these series of six animals, all died within eleven hours after the operation; the survival time was on the average definitely shorter than that found in the control series (1 and 2). The data are summarized in table 2.

The blood pressure showed a steady decrease despite repeated rises immediately following each administration of paredrine. The limb with the occluded vein showed a rapidly increasing edema with enlargement as high as 30 per cent above the control, amounting to 6.1 per cent of the body weight. The hematocrit showed a sharp increase in all instances. The rate of fluid accumulation and the amount lost into the limb were of the same order as seen in our previous untreated series (1 and 2).

*Discussion.* In our experiments there was no evidence of any benefit derived from this drug although its pressor effect was apparent. This negative effect may be peculiar to the form of shock studied and need not necessarily apply to other forms. In this type of shock, elevation of blood pressure transmitted to the capillaries by raising hydrostatic pressure would tend to increase the loss of plasma fluid, and so accelerate the development of the syndrome. Such an action would be expected with paredrine in all forms of shock in which escape of plasma fluid is a prominent feature. Only when the cause of shock is extensive vasodilatation with little loss of capillary permeability could paredrine or other pressor drugs be useful, since here in addition to raising the blood pressure and increasing the driving force to feed the heart, brain, etc., it would also shrink the vascular bed, and so restore venous return to the heart (12). Since plasma fluid loss due to increased capillary permeability seems to be an important feature of most, if not all forms of shock, the aggravation of this circumstance by increasing capillary hydrostatic pressure limits the value of such pressor drugs as paredrine to emergency measures while the more important procedure of replacing the lost plasma fluids is carried out. This latter therapy and the use of cortical substances which tend to check the progress of the mechanisms which make shock irreversible seem more suitable than mere blood pressure elevation.

## SUMMARY

1. Adrenal cortical extract (ACE) is a beneficial therapeutic agent in the treatment of shock following venous occlusion of a limb.

2. ACE shows a definite tendency to reduce the amount of fluid lost into the edematous limb, an action more striking than that of desoxycorticosterone acetate (DCA) alone. However, it has a less marked effect on survival than DCA.

3. Paredrine, a vasopressor substance, is of no benefit in shock in which the initiating factor is an escape of plasma fluid, since it appears to augment the escape of fluid through capillaries with impaired permeability.

We wish to express our indebtedness to Dr. L. N. Katz, at whose suggestion these studies were undertaken, for his guidance, and to Dr. K. Jochim for his suggestions in preparing this report.

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# THE DISTRIBUTION OF LACTIC ACID IN HUMAN BLOOD

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Almost all of the reported observations concerning the distribution of lactic acid between blood corpuscles and plasma indicate that lactate is present at higher concentrations in plasma than in blood cells (1, 2, 3, 4). However, Devadetta (5) found that this unequal distribution obtained only when total lactate concentration was above normal, whereas at low concentrations the distribution was similar to that of the other anions of blood. In previous investigations lactic acid concentration was varied by exercise (1, 5), anoxia (2, 3), and by the addition of lactate to whole blood *in vitro* (1, 5). The present study deals with the influence of glycolysis in human blood upon the concentrations of lactic acid in water of cells and serum.

PROCEDURE. All blood samples were obtained from three normal adult subjects under basal conditions. Venipuncture was accomplished without stasis after the forearm had been immersed in hot water for about 10 minutes in order to render the blood approximately arterial in composition (6). Aerobic defibrination was carried out promptly by stirring with a glass rod. Samples of the defibrinated whole blood were taken for determination of lactic acid, glucose, cell volume and water content. The remainder was either centrifuged at once to obtain serum for determination of lactic acid and water content, or incubated at 37° for one to six and a half hours, after which whole blood samples were taken and serum obtained by centrifugation for the determinations mentioned above.

*Analytical methods.* Lactic acid was determined by the method of Miller and Muntz (7) as modified by Barker and Summerson (8). Analysis of desaccharinized Somogyi filtrates of whole blood and serum were carried out in triplicate. No antiglycolytic agents were utilized in the procedures. It was found that no significant rise in blood lactate occurred during the period required for defibrination. Furthermore, lactic acid concentrations in defibrinated blood allowed to stand at room temperature for 15 to 20 minutes before precipitation of proteins never exceeded 14 mgm. per cent and often fell within the range of values for blood precipitated immediately.

Blood glucose was determined on Somogyi filtrates (9) by the method of Benedict (10).

Cell volume was measured with Daland hematocrit tubes as recommended by Eisenman, Mackenzie and Peters (11).

[1] This article represents work done in fulfilment of the thesis requirement for the degree of Doctor of Medicine at Yale University School of Medicine.

Water content was taken as the loss of weight of an aliquot of whole blood or serum dried to constant weight at 97.5°.

*Calculations.* Concentration of lactate in cells was calculated from concentrations in whole blood and serum by the formula:

$$L_c = \frac{L_b - L_s(1 - V_c)}{V_c}$$

TABLE 1

*The distribution of lactic acid between cells and serum during glycolysis*

| EXPERIMENT NUMBER | SUBJECT | DURATION OF INCUBATION | CELL VOLUME | | LACTIC ACID IN WHOLE BLOOD | | FINAL LACTIC ACID IN WATER OF | | DISTRIBUTION RATIO (1):(2) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | Initial | Final | Cells (1) | Serum (2) | |
| | | *hours* | *per cent* | *per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | |
| 1 | D. D. | 1 | 51.9 | 47.0 | 9.6 | 18.6 | 8.0 | 31.9 | 0.3 |
| 2 | D. D. | 1 | 49.4 | 47.5 | 7.0 | 21.0 | 14.5 | 32.9 | 0.4 |
| 3 | D. D. | 1 | 49.6 | 46.7 | 7.5 | 26.0 | 24.2 | 35.9 | 0.7 |
| 4 | E. B. D. | 1 | 43.1 | 38.7 | 7.8 | 20.4 | 20.3 | 25.7 | 0.8 |
| 5 | J. D. R. | 1 | 45.0 | 42.3 | 6.8 | 25.0 | 27.2 | 31.8 | 0.8 |
| 6 | J. D. R. | 1 | 41.0 | 42.1 | 9.7 | 28.0 | 31.6 | 34.6 | 0.9 |
| 7 | E. B. D. | 1 | 41.9 | 38.6 | 5.2 | 19.0 | 22.0 | 22.6 | 1.0 |
| 8 | D. D. | 1 | 51.1 | 47.9 | 6.5 | 23.2 | 27.9 | 28.4 | 1.0 |
| 9 | J. D. R. | 1 | 45.9 | 44.0 | 7.5 | 21.0 | 23.8 | 26.4 | 0.9 |
| 10 | D. D. | 1 | 49.9 | 48.0 | 7.6 | 25.0 | 36.8 | 25.5 | 1.4 |
| 11 A | J. D. R. | 5 | | 50.7 | | 70.6 | 60.4 | 104.5 | 0.6 |
| 11 B | J. D. R. | 6½ | | 50.1 | | 62.8 | 22.1 | 119.5 | 0.2 |
| 12 A | J. D. R. | 5 | | 47.0 | | 70.0 | 69.9 | 93.1 | 0.7 |
| 12 B | J. D. R. | 6½ | | 50.9 | | 80.4 | 64.2 | 122.0 | 0.5 |
| 13 A | D. D. | 5 | | 57.3 | | 62.0 | 39.6 | 112.4 | 0.4 |
| 13 B | D. D. | 6½ | | 58.6 | | 65.6 | 43.9 | 119.4 | 0.4 |
| 14* | D. D. | 5 | 47.7 | 50.2 | 6.3 | 74.0 | 71.6 | 101.9 | 0.7 |
| 15* | D. D. | 5 | 45.7 | 50.2 | 10.3 | 82.8 | 90.0 | 104.7 | 0.9 |
| 16* | J. D. R. | 5 | 51.0 | 57.4 | 8.0 | 84.4 | 74.3 | 129.9 | 0.6 |
| 17* | J. D. R. | 5 | 51.7 | 53.3 | 12.0 | 80.4 | 59.0 | 129.5 | 0.5 |

* Glucose added before incubation. Initial glucose concentrations: experiment 14, 172; experiment 15, 163; experiment 16, 199; experiment 17, 207 mgm. per cent.

in which $L_c$, $L_b$, and $L_s$ are lactate concentrations in cells, whole blood and serum, respectively, and $V_c$ is the cell volume.

The concentration of water in cells was calculated from the water of whole blood and of serum by an analogous formula.

RESULTS. Ten measurements of the lactic acid concentration in blood drawn under basal conditions and deproteinized immediately after defibrination, gave values varying from 5.2 to 9.7 mgm. per cent. These values are in good agreement with basal values reported in the more recent literature. In 5 of 6 instances in which lactic acid concentrations in water of cells and serum were compared under basal conditions, the two concentrations were identical within the limits of error of the analytical procedures. In a single experiment the concen-

tration in cell water was 16.5 mgm. per cent, while that in serum water was only 11.6 mgm. per cent. With this single exception, lactic acid was found to be evenly distributed through the water of cells and serum under basal conditions. These observations confirm those of Devadetta (5).

Concentrations of lactic acid in cell water and serum water after glycolysis has been allowed to proceed at 37° for 1, 5 and 6½ hour periods are given in table 1. After glycolysis of one hour's duration a tendency for serum lactate to exceed the concentration in cells is already apparent and this inequality of distribution is more marked and more consistent at the end of the longer periods.

Increments in whole blood lactate concentration and decrements in blood glucose concentration during incubation periods of 1 and 5 hours are compared in table 2. Except in experiment 7, in which an unusually small decrease in

TABLE 2

*The relation of lactic acid production to glucose destruction in blood during glycolysis*

| EXPERIMENT NUMBER | SUBJECT | DURATION OF INCUBATION | DECREASE IN BLOOD SUGAR | INCREASE IN BLOOD LACTATE | GLUCOSE CONVERTED TO LACTIC ACID |
|---|---|---|---|---|---|
| | | *hours* | *mgm. per cent* | *mgm. per cent* | *per cent* |
| 1 | D.D. | 1 | 15 | 9.0 | 60 |
| 2 | D. D. | 1 | 24 | 14.0 | 58 |
| 3 | D. D. | 1 | 24 | 18.5 | 75 |
| 8 | D. D. | 1 | 24 | 16.7 | 70 |
| 10 | D. D. | 1 | 19 | 17.4 | 94 |
| 5 | J. D. R. | 1 | 30 | 18.2 | 61 |
| 9 | J. D. R. | 1 | 23 | 13.8 | 60 |
| 6 | J. D. R. | 1 | 24 | 18.3 | 76 |
| 4 | E. B. D. | 1 | 14 | 12.6 | 90 |
| 7 | E. B. D. | 1 | 8 | 13.8 | 174 |
| 14* | D. D. | 5 | 81 | 67.7 | 83 |
| 15* | D. D. | 5 | 85 | 72.8 | 86 |
| 16* | J. D. R. | 5 | 107 | 76.4 | 71 |
| 17* | J. D. R. | 5 | 95 | 68.4 | 72 |

* Glucose added before incubation.

blood glucose was observed, only 58 to 94 per cent of the glucose which disappeared could be accounted for as increased lactate.

A decrease in cell volume was frequently observed at the end of 1 hour's incubation (table 1). In experiment 6 an insignificant rise was observed (1.1 vols. per cent). In experiments 2, 9 and 10 a probably significant fall occurred (2.7 to 4.9 vols. per cent). After 5 hours' incubation no significant net change in cell volume occurred in two experiments whereas in two others cell volume increased by 4.5 and 6.4 vols. per cent.

Discussion. Blood lactic acid at the normal basal value is equally distributed through the water of cells and serum. When, however, the lactic acid concentration is raised, whether by addition of lactate (1, 5), exercise (1, 5), anoxia (2, 3), or by glycolysis, the concentration in serum water almost always exceeds

that in cell water. This unequal distribution cannot be attributed to restraint of diffusion exerted by the serum colloids since lactate added to suspensions of blood cells in saline is distributed just as it is in whole blood (5). Devadetta (5) has also shown that lactate ion can cross the corpuscular membrane in either direction. The validity of these observations is supported by the ability of lactate to leave the cells and to enter the serum during glycolysis. The partition of lactate cannot be dependent upon the glycolytic process since procedures which prevent glycolysis, such as addition of fluoride or washing the blood corpuscles, do not alter the distribution of lactic acid (5). It is not improbable, however, that the glycolytic process does modify lactate partition to some extent. Whereas in Devadetta's experiments, in which glycolysis played no role, a good correlation existed between the distribution ratio of lactate and the total lactate concentration, in the present study there is only a fair correlation between these variables. Furthermore, in one experiment (no. 11) a large amount of lactate entered the serum from the cells in the period just after glycolysis was completed and without any further rise in total lactate. Since it is known that other ions (12) are transferred during glycolysis, it seems not improbable that the lactate partition expected on the basis of concentration alone might be altered in response to the metabolic requirements of the cells.

Failure to recover lactic acid in sufficient quantity to account for the decrease in blood sugar during glycolysis may be in part due to oxidative metabolism of glucose by the white blood cells. However, the amount of glucose which could disappear by this route is probably too small to account for the entire deficit observed. Furthermore, quantitative conversion of glucose to lactic acid may not occur even when oxidative processes are inhibited (13).

This disparate distribution must have some bearing on data dealing with lactic acid equilibria in the living organism. Obviously, if there is such an equilibrium it must be between the concentrations in the tissues and the plasma, not whole blood. After exercise or in other conditions in which lactic acid is elevated, its concentration may be far lower in whole blood than in plasma.

Whether other cells behave like blood cells is not known. Ghaffar (14) found that lactate added to frog muscle tissue distributed itself through a volume of fluid approximating that of the extracellular space only. Lactic acid produced in the muscle appeared to diffuse out freely; but even when none was added, the concentration was greater in extracellular fluid than in the muscle cells. Devadetta (15), in similar experiments, estimated that lactic acid diffused into all the water of muscle. Newman (16) found that the concentrations of lactic acid in blood and muscle taken from rats both before and after exercise differed by no more than the experimental error. Gesell et al. (2) and Eggleton and Evans (4) have analyzed plasma and muscles under various conditions. Although on the average concentrations in the two media agreed, in individual observations there were divergences in both directions. There was some tendency for the concentrations to be greater in the plasma when lactic acid was elevated, the disparities in some instances being too great to be attributed to differences in the amounts of water in the two media. Determination of the distribution of lactate in the

tissues is an extremely difficult matter, since the acid is so ubiquitously produced and so continually removed, and since production does not cease when the tissue is abstracted for analysis. Since, in the animal lactic acid originates in the muscle, if it enters the blood by a simple process of diffusion, it should, as it rises, be more concentrated in muscle than in plasma. Those experiments in which the concentration gradient is in the opposite direction, from plasma to muscle, must, therefore, be given extra weight.

Danowski (12) in experiments on the transfer of potassium between blood cells and plasma found that at the end of 5 hours' incubation the volume of the cells had not changed. In a single instance he noted some contraction of the cells after 2.5 hours. In the present experiments the volume of the cells decreases distinctly in the course of the first hour, but has been again restored at the end of 5 hours. The reactions associated with this transitory transfer of water remain to be discovered.

## SUMMARY

The distribution of lactic acid between blood cells and plasma during spontaneous glycolysis has been investigated. In the blood of humans at rest in the post-absorptive state, lactate is evenly distributed through the water of cells and serum. As the concentration of lactic acid rises with glycolysis, the ratio, lactic acid in water of serum: lactic acid in water of cells, steadily increases. This is in agreement with Devadetta's observations of the distribution of lactic acid at rest, during exercise and after the addition of lactate.

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# INFLUENCE OF PROPRIOCEPTIVE VAGAL AFFERENTS ON PANTING AND ACCESSORY PANTING MOVEMENTS IN MAMMALS AND BIRDS

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Since the important original investigations of Hering and Breuer in 1868 on the influence of the vagi on respiration, subsequent evidence has increased our knowledge of their significance. The modern concept is essentially that of a drive mechanism which together with the drive of higher centers (e.g., the "pneumotaxic" center of Lumsden) maintains respiratory rhythmicity. The vagal effects have been shown to be essentially inhibito-inspiratory or excito-expiratory resulting in a more shallow and consequently faster type of breathing. The biological significance of such an arrangement must be prevention of excessively great variations in alveolar oxygen and carbon dioxide pressures thus approaching more nearly a condition of homeostasis.

Among mammals and especially in birds vagotomy results in decreased breathing rates. Vagotomy in the fowl results in an alarming decrease in rate of pulmonary ventilation such that the bird becomes cyanotic. Inspirations occur as infrequently as four per minute. During successive days the rate increases somewhat. Cocainization of the vagi produces effects identical with vagotomy.

It has been stated (Pitts, Magoun and Ranson, 1939) that rhythmicity (in the cat) is imposed upon the medullary centers by two bilateral mechanisms: one a vagal mechanism, the other a pneumotaxic mechanism, the center for which is located in the upper few millimeters of the pons. If the connections of both these mechanisms with the respiratory centers are lost, respiratory rhythmicity is interrupted and in its place a tonic, maintained inspiration ensues. If, however, there remains one vagus intact, or a part of one descending pneumotaxic connection with the respiratory center, its action remains rhythmical. Hong and Nicholson (1942) report that in decerebrate dogs with mid-pontine transection intermittent central vagal stimulation may cause normal rhythmic breathing at a frequency corresponding to that of the stimulation. Nicholson and Hong (1942) showed that section through the middle or upper pons in the anesthetized (morphine-urethane, pentobarbital or ether) dog commonly resulted in respiratory arrest and death. In the non-anesthetized dog, however, similar section produced only deep held inspirations with superimposed rhythmical respiration at the normal rate. Pitts (1942) also shows that transection of the brain through the middle or lower pons plus bilateral vagal section is followed by apneusis. Stella (1939) has shown that the pneumotaxic connections with the apneustic center are bilateral and are mainly homolateral.

We have found that panting can occur in mammals after bilateral vagotomy and in fact appears entirely normal (fig. 1, A). If one vagus is cut (or cocainized)

in the panting bird no visible change occurs, but if the other vagus is then sectioned (or cocainized) panting stops at once (fig. 1, B, C). Even though the body temperature is well above the panting threshold, panting immediately

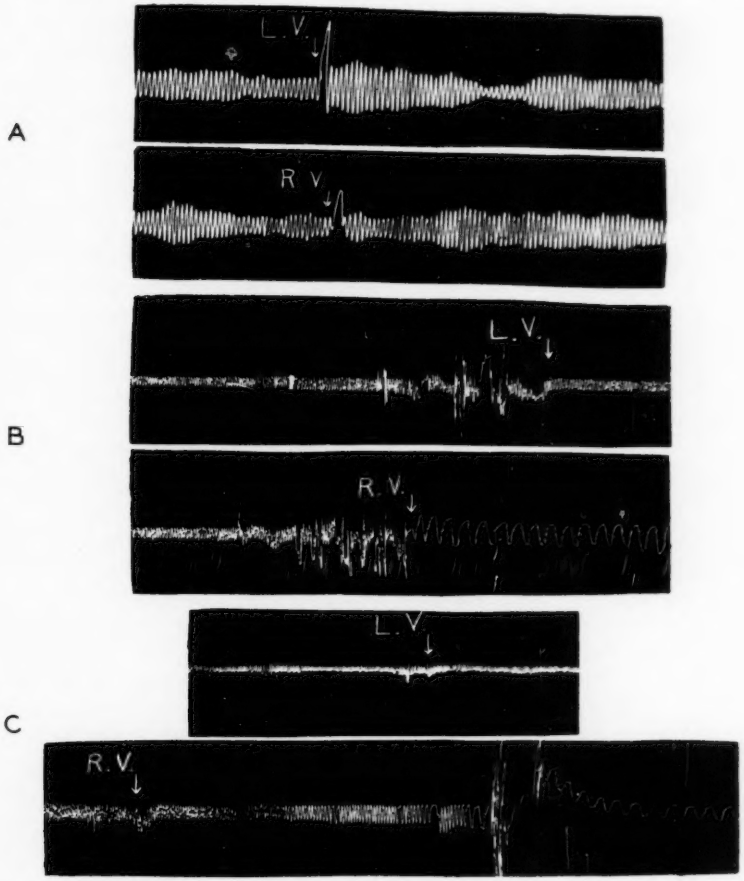


Fig. 1. A. Record of panting in the rabbit. Section of left and right vagus indicated by arrows. B. Panting in the chicken. Section of left and right vagus indicated by arrows. C. Panting in the chicken. Application of cocaine hydrochloride (1.0 per cent solution) to left and right vagus shown by arrows. Note no change in rate after cocainization of left vagus but an effect taking place quantitatively as separate fibers of the right vagus become blocked.

ceases when the second vagus is sectioned. Nor does it matter in which order section of the two vagi occurs, either left or right may be sectioned first with no apparent effect on the panting rhythm. This raises an interesting speculation. Why should no change in panting rate result from unilateral vagotomy while

abrupt cessation results from double vagotomy? It appears that either vagus is more than an ample pathway for the Hering-Breuer fibers and likewise that both are functioning submaximally as regards the number of fibers at all times. One might imagine an analogue to a group of wires in a cable supplying an electric lamp. If half of the wires are cut the lamp will continue to glow without reduced intensity if the remaining wires are still ample to conduct the necessary current.

Examination of figure 1, B will show the continuation of panting with no reduced rate after section of the left vagus but a prompt abolition after the remaining vagus has been cut. No such effect can be found in the panting rabbit either with cocainization or surgical severance of the vagi.

During panting in birds and mammals there occur certain accessory movements together with salivation. In the rabbit the nose-flap can be seen to move with each breath. Tracheotomy has no effect on this motor response. In the bird accessory panting movements consist of opening and closing the beak and raising and lowering the tongue. Tracheotomy and the resulting "sham panting" do not influence those motor responses. Salivation is more apparent in the tracheotomized panting mammal or bird due to the absence of the air-stream in the naso-pharynx.

At attempt to study the effect of the stretch receptors of the lungs on the panting rhythm in birds and mammals was carried out by artificial distention of the lungs via a tracheal cannula and by artificial respiration induced by bellows action at a rate slower than that of panting. Emphysema was produced by attaching a rubber hose to the tracheal cannula and forcing air into it during panting thus stimulating the stretch receptors during polypneic breathing. With the rabbit distension of the lungs caused temporary cessation of panting. As soon as the pressure was relieved the polypneic rhythm returned. It should be mentioned that if the distention of the lungs was maintained for a length of time the panting movements returned probably due to an accumulation of carbon dioxide or to an increase in sensitivity of the centers or to both.

With the non-anesthetized duck distention of the lungs either by a steady positive pressure or intermittently by bellows action caused no change in the panting rhythm. However, in the duck anesthetized with a barbiturate (sodium amytal) distention of the lungs during panting caused slowing of the polypneic rhythm which became progressively slower with maintained distention until complete apnea occurred. This points to the fact that barbiturate narcosis can modify the normal respiratory regulation which has been reported by others (Beecher and Moyer, 1942; Nicholson and Hong, 1942) and was shown by us in previous work (Randall and Hiestand, 1939). It shows that during amytal anesthesia the relationship of the stretch receptors to the respiratory centers, e.g., the panting center, is interfered with.

By comparison of the bird and mammal we thus see that distention of the lungs of the rabbit is more effective in reducing the rate of polypneic breathing than in the bird but at the same time we must acknowledge the greater significance of the proprioceptive vagal drive of the bird because panting is impossible

without it. It seems paradoxical that emphysema should not slow or abolish breathing in the bird if the vagal drive here is more powerful. One imagines the "vagal drive" as an inhibito-inspiratory mechanism (and possibly inhibito-expiratory) that depends upon stretch (or collapse) of the lungs for its stimulus. Why then should panting continue unabated in the rabbit and cease altogether in the bird after vagotomy? It must be that the rabbit depends upon a panting drive from a "higher" brain center entirely whereas the bird depends mainly upon the vago-proprioceptive drive or vago and pneumotaxic drives.

In the mammal section of one vagus reduces the breathing rate; section of the remaining vagus reduces it still further, and yet the vagotomized rabbit can pant normally. In the bird section of one vagus reduces the breathing rate considerably more than in the mammal; section of the other vagus almost produces respiratory arrest. The vagotomized bird can not pant. If the vagal drive in birds is so much greater than in mammals why does emphysema reduce breathing in the mammal but not in the bird? Perhaps the stimulus of emphysema is entirely different from the normal lung-stretch breathing stimulus, or possibly different types of receptors exist in the lungs.

## CONCLUSIONS

1. Stimulation of the pulmonary stretch receptors by emphysema in the rabbit during panting progressively reduces the panting rate and results in apnea if prolonged.

2. No such effect occurs in the non-anesthetized bird during panting.

3. Under barbiturate anesthesia stimulation of the stretch receptors results in slowing of the panting rate in birds similar to that of the non-anesthetized mammal.

4. Section (or narcotization) of either or both of the vagi has no effect on panting in mammals.

5. Section (or narcotization) of either of the vagi of birds has no effect on panting.

6. Section (or narcotization) of both vagi of birds immediately abolishes panting regardless of the order of sectioning.

7. The vagal proprioceptive drive is relatively more significant in respiratory regulation in birds than in mammals.

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# RELATION BETWEEN PULMONARY VENTILATION AND OXYGEN CONSUMPTION AFTER EXERCISE

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The cardio-respiratory system is the most important limiting factor in the performance of strenuous muscular exertion by normal men. Measurements of its functions have given a reasonably complete analysis of the mutual relationships of its component parts during exercise (see, for example, the work of Boothby (3) and of Bock, Dill and their colleagues (2)). Somewhat less attention has been paid by physiologists to the cardio-respiratory functions in recovery after exercise than during exercise. For this reason, we decided to study in recovery after exercise the cardiac output, the blood lactate, oxygen consumption, pulmonary ventilation and pulse rate in order to find out if it is possible to relate them one with another and at the same time to see whether the individual variations from subject to subject are indicative of variations in general physical fitness. The few reports in the literature pertinent to this particular study will be mentioned in the discussion of our results.

EXPERIMENTAL PROCEDURE. Two types of experiments were carried out, differing in the grade of exercise chosen and the measurements made during recovery.

1. The subject either ran on a motor-driven treadmill at 7 miles per hour and 8.6 per cent grade until exhausted or else walked at 3.5 miles per hour on the same grade until he reached a steady state. He then sat quietly during recovery. During the entire experiment the ventilation was measured by collecting the expired air in a large Tissot gasometer and the oxygen consumption was measured from samples drawn at frequent intervals from a mixing chamber inserted between the subject and the gasometer.

2. In the second type of experiment the exercise was always a walk at 3.5 miles per hour with the grade at 7.5, 8.6 or 9.5 per cent. Ventilation and oxygen consumption were determined by collection and analysis of expired air at only three points: *a*, during the steady state; *b*, between three and five minutes of recovery; *c*, at 20 minutes in recovery. At the same time the cardiac output was measured by Grollman's (5) acetylene rebreathing method, and the lactate in the blood from a finger prick was determined by Edwards' (3) modification of the method of Friedemann, Cotonio and Shaffer (4).

RESULTS AND DISCUSSION. Figures 1, 2 and 3 depict the blood lactate, the pulmonary ventilation, the pulse rate, the cardiac output, and the oxygen consumption following two grades of exercise. In each case the scales are

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chosen and placed so that all the curves start at a common point which represents the value of that variable in the steady state of the exercise. In this way, although actual values are plotted, the same type of curve is obtained as if the measurements were calculated as percentages of the values at steady state. It will be seen that all of the variables decrease, and in order of slowness fall in

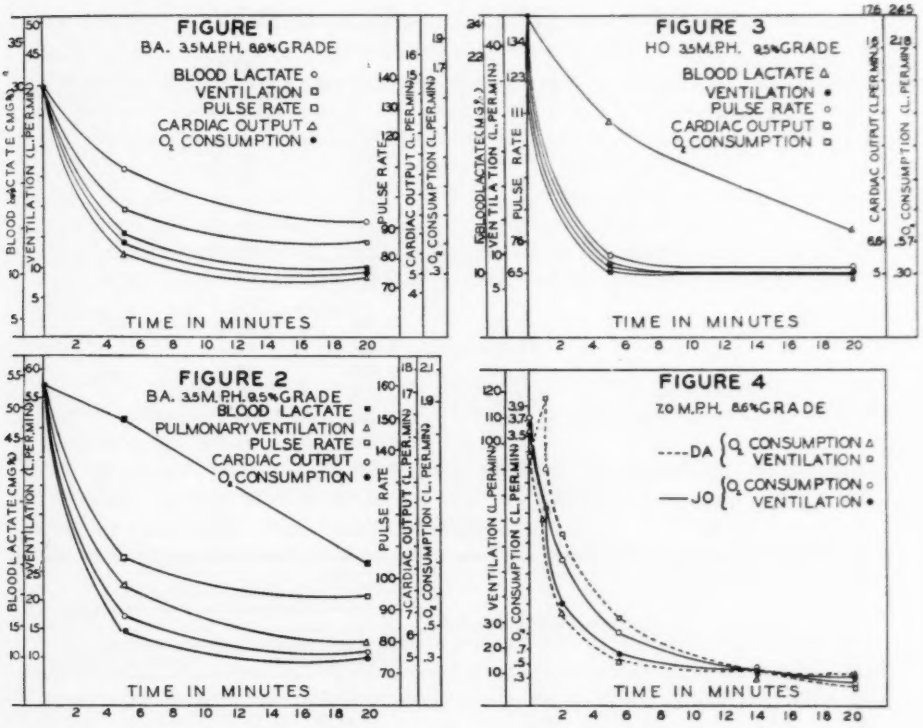


Fig. 1. Oxygen consumption, pulmonary ventilation, blood lactate, and cardiac output in subject *Ba* after walking 12 minutes at 3.5 miles per hour and 8.6 per cent grade.

Fig. 2. The same measurements on the same subject walking at 3.5 miles per hour and 9.5 per cent grade.

Fig. 3. The same measurements on the subject *Ho* after walking 12 minutes at 3.5 miles per hour and 9.5 per cent grade.

Fig. 4. Oxygen consumption and pulmonary ventilation of subjects *Da* and *Jo* after running to exhaustion at 7.0 miles per hour and 8.6 per cent grade.

the following order: lactate, ventilation, pulse rate, oxygen consumption and cardiac output. The last two follow one another very closely, which finding agrees with that of Bansi and Groscurth (1) on normal men as contrasted with cardiac patients. Likewise the relations between pulse rate and oxygen consumption are in confirmation to the work of Lythgoe and Pereira (7).

The special relation between ventilation and oxygen consumption can be

shown best by examining the ratio oxygen consumption/pulmonary ventilation, the ventilatory efficiency. Table 1 shows repeated measurements of this value

TABLE 1

*Ventilation and oxygen consumption during recovery after 15 minutes' walking uphill at 3.5 m.p.h. and 8.6 per cent grade*

| SUBJECT | TIME IN RECOVERY | PULMONARY VENTILATION | OXYGEN CONSUMPTION | VENTILATORY EFFICIENCY | BLOOD LACTATE |
|---|---|---|---|---|---|
| | *minutes* | *l. per min. S.T.P.* | *l. per min. S.T.P.* | OXYGEN CONSUMPTION / PULMONARY VENTILATION | *mgm. in 100 ml. of blood* |
| De | 0– 1 | 34.92 | 0.701 | 20.1 | 40 |
| | 1– 3 | 20.02 | 0.404 | 20.0 | |
| | 3– 5 | 18.62 | 0.402 | 21.6 | 32 |
| | 5– 8 | 16.60 | 0.391 | 24.0 | |
| | 8–10 | 15.36 | 0.350 | 22.8 | |
| | 10–12 | 13.00 | 0.341 | 26.2 | |
| | 12–15 | 13.65 | 0.340 | 24.9 | 22 |
| Ho | 0– 1 | 14.88 | 0.495 | 33.2 | 18 |
| | 1– 3 | 13.48 | 0.430 | 31.9 | |
| | 3– 5 | 12.00 | 0.405 | 33.7 | 16 |
| | 5– 8 | 10.40 | 0.313 | 30.0 | |
| | 8–10 | 9.50 | 0.280 | 29.6 | |
| | 10–12 | 8.52 | 0.260 | 30.6 | |
| | 12–15 | 7.00 | 0.260 | 37.2 | 12 |

TABLE 2

*Individual variations in ventilatory efficiency compared with physical fitness for hard work*

| SUBJECT | INDEX OF PHYSICAL FITNESS* | VENTILATORY EFFICIENCY OXYGEN CONSUMPTION/PULMONARY VENTILATION | |
|---|---|---|---|
| | | Ten minutes after moderate exercise | Ten minutes after exhausting exercise |
| Ho | 95 | 41.5 | 28 |
| Sw | 88 | 41.3 | |
| Do | 78 | 39.8 | |
| Jo | 73 | | 25.6 |
| Wit | 63 | | 24.6 |
| Mo | 55 | 35.7 | 24.3 |
| Fo | 52 | 35.7 | 24.1 |
| Fa | 47 | 33.3 | 24.1 |
| He | 41 | | 24.3 |
| Ba | 22 | 28.6 | 21.7 |
| Da | 22 | | 20.9 |
| De | 16† | 21.6 | |

* According to the test of Johnson, Brouha and Darling.

† Measured by other criteria.

in recovery after moderate exercise, and figure 4 the pulmonary ventilation and oxygen consumption of two subjects after exhausting exercise. The dissociation between the curves for ventilation and oxygen consumption is more marked

after severe than after moderate exercise, and as shown in table 1, is associated with differences in blood lactate. The results lend themselves to the interpretation that ventilation is maintained relatively higher than oxygen consumption by increased acidity of the blood.

On examining the data for different subjects, considerable variation is found in the ventilatory efficiency after work is over which might logically be related to the physical fitness of the individuals. Table 1 shows that for a subject (De) known to be in poor physical condition, the ventilatory efficiency is poor at all times after moderate exercise, and that for a subject known to be in good physical condition (Ho) it is good throughout. Table 2 lists twelve subjects in order of their physical fitness for hard work as measured by the test of Johnson, Brouha and Darling (6). It will be seen that in this small number of subjects there is a good correlation between the subject's general physical condition for hard work and his ventilatory efficiency in recovery after exercise. Estimation of ventilatory efficiency is hardly practicable for routine testing of physical fitness, but might be useful clinically in following the progress of patients with pulmonary and cardiac disease.

## SUMMARY

1. The relations among pulmonary ventilation, oxygen consumption, cardiac output, pulse rate, and blood lactate have been studied in the recovery period after exercise.

2. The rates of decrease of oxygen consumption and cardiac output are almost the same; the rate of decrease of the pulse rate is slightly slower; that of pulmonary ventilation is even slower; that of the blood lactate is the slowest of all.

3. It is suggested that the deviation of the curve for ventilation is caused by a continued stimulus to ventilation in the blood stream, as shown by the blood lactate.

4. The ventilatory efficiency as expressed by the ratio oxygen consumption/pulmonary ventilation is less after exhausting than after moderate exercise, and shows considerable variation from subject to subject after the same amount of work.

5. In twelve subjects, the ventilatory efficiency correlates well with their established general physical fitness for hard muscular work.

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# METABOLIC EFFECTS OF LOCAL ISCHEMIA DURING MUSCULAR EXERCISE

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The method of Jarisch and Gaisböck (11) of studying the effect of ischemia of the legs and arms furnishes a useful technique for measuring various aspects of metabolism. These authors as well as Asmussen, Nielsen and Christensen (1) studied its effect upon oxygen consumption.

The same technique is utilized in this paper to measure at the same time the changes in lactic acid. On the general subject of lactate metabolism, Hill (9) and associates first studied intensively the lactate concentration in blood following muscular exercise, the rate of its removal from the blood stream and its relation with oxygen consumption and oxygen debt. Margaria, Edwards and Dill (14) established that the removal of lactate from the body of a man resting after exercise is proportional to the amount that is present at any given time. Newman and his associates (17) observed that the rate of removal of lactate in exercise increases in almost direct proportion to the metabolic rate up to some critical level, different for each subject. Recently, Robinson (18) has studied the rate of removal of lactate in exercise and rest and its relation to the oxygen debt, with subjects performing successively decreasing amounts of work and has found that the rate of removal of lactate is higher during work and that the extra oxygen consumption for lactate removal is lower than at rest.

METHODS. A. *Analytical methods.* Analysis of the samples of expired air was made with a Haldane gas analysis apparatus. Blood lactates were measured by Edwards' (3) modification of the method of Friedemann, Cotonio and Shaffer (7).

B. *Experimental procedure.* The experiments consisted in cutting off the limb's circulation while muscular work was being carried out, and in relieving the pressure after a period of work in order to liberate into the body the metabolites produced locally by the limb's activity. In effect, therefore, the conditions were analogous to making a sudden injection into the general circulation of these accumulated metabolites, making possible a study of lactate diffusion and removal, and its relation with oxygen debt.

Two fundamental types of experiments were performed:

1. *Experiment allowing recovery at rest.* The subject, well trained for the procedure, walked on a motor-driven treadmill at 3.5 m.p.h., 8.6 per cent grade, until a steady state was reached (usually within 12 min.). Then, by means of sphygmomanometer cuffs, the circulation of both legs was rapidly cut off just at the distal insertion of the gluteal muscles.

[1] Rockefeller Fellow.

[2] Pan American Fellow.

After an appropriate interval, usually about one and one-half minutes, the pressure was instantaneously relieved and the subject rested for 20 minutes or longer. Samples of capillary blood and of expired air were taken before, during and after the ischemia.

2. *Experiment allowing recovery during continued walking.* The second type of experiment was carried out in the same way, the only difference being that after release of the ischemia, the subject, instead of resting, continued to walk on the treadmill at the same rate and grade as before.

C. *Determination of the muscle mass.* The muscular mass was estimated by measuring the volume of water displaced by immersion of the leg. We have assumed that 70 per cent of the mass of the legs is muscle (Froshe and Fraenkel, and Vierords Tabellen cited by Jarisch and Gaisböck (11)) and that the muscle of the legs accounts for 43 per cent of the total muscle of the body.

TABLE 1

*Effects of ischemia of both legs during walking uphill at a grade of 8.6 per cent*

| SUBJECT | SPEED OF WALK | CONDITION OF CIRCULATION OF LEGS | PULMONARY VENTILATION | OXYGEN CONSUMPTION | R.Q. |
|---|---|---|---|---|---|
| | *m.p.h.* | | *l. per min.* | *l. per min.* | |
| Bar | 3.5 | Normal | 43.7 | 1.525 | 0.90 |
| | | Ischemic | 38.2 | 1.146 | 0.92 |
| | | First min. after ischemia | 55.4 | 1.950 | 1.12 |
| Bar | 2.4 | Normal | 36.6 | 1.320 | 0.84 |
| | | Ischemic | 33.0 | 1.062 | 0.82 |
| | | First min. after ischemia | 50.0 | 1.750 | 1.19 |
| Con | 3.5 | Normal | 48.0 | 2.425 | 0.89 |
| | | Ischemic | 43.0 | 1.680 | 0.90 |
| | | First min. after ischemia | 70.5 | 3.250 | 1.09 |
| Con | 2.4 | Normal | 38.6 | 1.941 | 0.85 |
| | | Ischemic | 35.4 | 1.344 | 0.95 |
| | | First min. after ischemia | 52.6 | 2.250 | 1.10 |

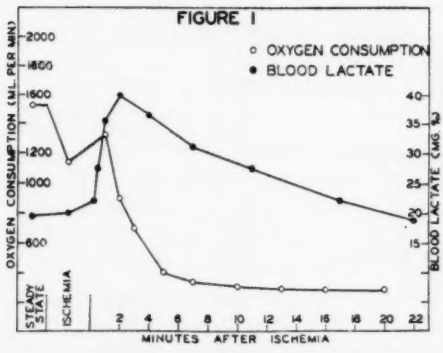
RESULTS AND DISCUSSION. Subjectively, the subjects noticed during the ischemic period, progressive difficulty in walking because of numbness, stiffness, and at the end of the period, usually cramps, inability to move and pain in almost every muscle of the leg. Upon relief of the ischemia the subject noticed within a few seconds the following symptoms in order of appearance: *a*, increased ventilation; *b*, palpitation of the heart; *c*, outbreak of profuse cold sweating. These symptoms reached their greatest intensity usually within fifteen seconds and then rapidly subsided.

1. *Oxygen consumption and ventilation.* The effects of ischemia during walking at two different rates are shown in table 1 for two subjects. In agreement with the work of Asmussen (1) there was during ischemia a diminution of the oxygen consumption. In addition we found a diminution of ventilation, and no change in the R.Q. During the first minute after ischemia there was a large increase in ventilation, oxygen consumption and R.Q.

The oxygen consumption of the leg muscles was calculated according to the method of Asmussen, Nielsen and Christensen (1), who assume that the difference between the oxygen consumption in the steady state and in the ischemic state represents the oxygen consumption of the legs. For one subject walking at 3.5 m.p.h. and 8.6 per cent grade the oxygen consumption of the leg muscles was thus calculated to be 38.7 cc. per kilogram and minute, and per kilogram-meter of work done by the body was 0.096 cc. per kilogram of leg muscle.

The cause of the sudden change in ventilation and oxygen consumption upon release of ischemia is apparently the sudden influx into the general circulation of the metabolites of the working ischemic muscles.

The relations among ventilation, oxygen consumption and blood lactate are shown in table 2 and in figures 1 and 2 for the period following release of ischemia. Typical experiments are shown in which the subject sat down im-



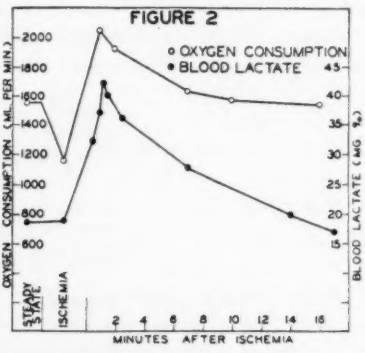




Fig. 1. Oxygen consumption and blood lactate in typical experiment on subject *Ba* during and after release of total ischemia of both legs. The subject began to rest at the same time as ischemia was relieved.

Fig. 2. The same measurements as in figure 1. The subject continued walking after release of the ischemia.

mediately after release of the ischemia and in which the subject continued to walk. The increases in ventilation and in oxygen consumption are proportional to the amount of lactate released into the general blood stream. In the walking experiments, the ventilation and oxygen return to their control levels within ten minutes after release of the ischemia; in the resting experiments, within twenty minutes.

Further discussion of the changes in ventilation in relation to the work of Harrison (8) will be reserved for another paper.

II. *Production of lactate by the muscles during activity.* After release of the ischemia, lactate rapidly diffused into the blood stream as shown in figures 1 and 2. Its maximal value after the release of ischemia was reached in about $1\frac{1}{2}$ minutes when the subject continued walking, and in about 2 minutes when he stopped walking at the time ischemia was relieved. The time spent for lactic acid to reach its equilibrium in blood can be attributed to the time neces-

sary for diffusion of the lactic acid from muscles to blood and into the idle tissues. This period ought to be shorter in exercise than in rest, because of the increased blood flow.

We have computed the quantity of lactate produced by a given muscle mass during a given amount of work making an approximation after Jarisch and Gaisböck (11) of the mass of muscle with the circulation arrested and the increase of lactic acid in the blood when the circulation was restored. For this computation we have assumed the lactate to be uniformly distributed in the fluid of the body, as shown by Margaria and Edwards (13) and by Newman (16). The lactate production under the conditions of our experiments increases with faster rate of work (fig. 4) and the muscular mass (fig. 5).

III. *Removal of lactate.* Data concerning the removal of lactate from the blood stream at rest and during work are shown in table 2 and in figures 1 and 2. In agreement with the equation of Margaria and Edwards (13) three facts

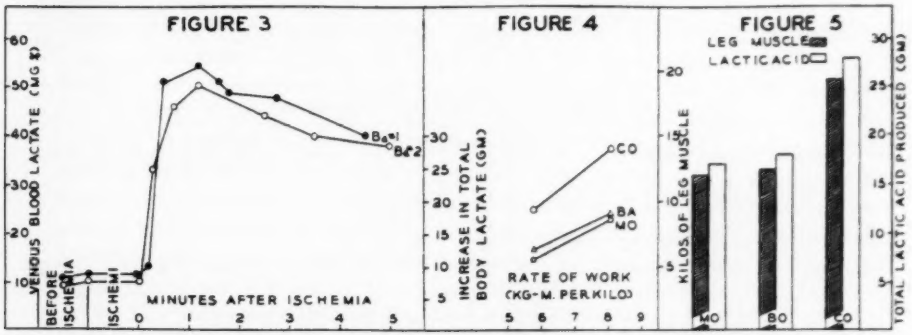



Fig. 3. Diffusion of lactate into the local venous blood before, during and after total ischemia of the exercising arm.

Fig. 4. The lactate production during ischemia of the legs in relation to rate of work.

Fig. 5. The total lactate production in relation to the bulk of the leg muscles.

worthy of consideration are: *a.* The lactate removal is an exponential function expressed approximately by the equation, $y = ae^{-kx} + b$; where $a$ is a constant equivalent to the maximal lactate value minus the constant $b$; $b$ is the lactic acid concentration at rest in the resting experiment and also represents the lactate at steady state in the walking experiment; $k$ is the velocity constant that expresses how $a$ approaches $b$ through time "$t$". *b.* The value of $k$ is much larger when the lactate is removed during continued walking than during rest. This means that the rate of removal is much higher in activity than at rest, in agreement with Newman et al (17). The lactic acid eliminated in the sweat and urine (20, 21) and used by the heart for fuel are not enough to explain this large difference.

IV. *Relation between removal of excess lactate and oxygen consumption.* Of eleven experiments in which the subject rested after the ischemia, and ten in which he continued walking, examples are shown in table 2 and in figures 1

and 2, from which it can be inferred: *a*, that the amount of lactate removed from the body per liter of oxygen is much greater during work than during rest (Robinson has found the same thing in experiments quite different in procedure from ours); and *b*, that the rate of removal of lactate does not alter after all of the extra oxygen has been consumed, and the oxygen consumption is once more at its basal level in the resting experiments, or its value for the steady state in the working experiments (figs. 1 and 2). This fact may indicate that removal of lactate and extra oxygen consumption are independent.

The relation among lactic acid production, lactic acid removal and oxygen utilization in muscle has been carefully studied. In isolated muscle lactic

TABLE 2

*Relation between oxygen consumption and disappearance of lactate after ischemia of the legs*

| TIME AFTER RELEASE OF ISCHEMIA | PULMONARY VENTILATION | OXYGEN CONSUMPTION | R.Q. | LACTATE | | | | OXYGEN DEBT | LACTATE REMOVED PER LITER OF $O_2$ DEBT |
|---|---|---|---|---|---|---|---|---|---|
| | | | | In blood | Total in body | Amount removed from body calculate from 0 min. | Rate of removal of lactate | | |
| Subject "Co" resting after ischemia | | | | | | | | | |
| *minutes* | *l./min.* | *l./min.* | | *mgm./100 ml.* | *grams* | *grams* | *Velocity constant "K"* | *liters* | *grams* |
| 0 | 49.2 | 1.780 | 1.23 | 46 | 36.8* | | | | |
| 2 | | | | 40 | 32.0* | | | | |
| 7 | 14.40 | 0.496 | 0.98 | 30 | 24.0* | | | | |
| 20 | 8.30 | 0.343 | 0.84 | 22 | 17.6* | 19.2 | 0.062 | 2.460 | 7.80 |
| Subject "Co" continuing to walk after ischemia | | | | | | | | | |
| 0 | 70.40 | 3.170 | 1.05 | 45.2 | 36.1* | | | | |
| 1½ | | | | 41 | 32.9* | | | | |
| 5 | | | | 30 | 24.0* | | | | |
| 7 | 52.00 | 2.480 | 0.87 | | | | | | |
| 10 | | | | 25 | 20.0* | | | | |
| 15 | 53.70 | 2.470 | 0.92 | | | | | | |
| 17 | | | | 18 | 15.2* | 20.9 | 0.124 | 1.150 | 17.4 |

* Calculated.

acid formed anaerobically can in the presence of oxygen be oxidized or resynthesized into its precursor (5) about one mol of lactic acid being burned to supply energy for resynthesis of 4 mols (15). The rate of resynthesis is to some extent dependent upon the severity of the previous exercise (2). In muscles with intact circulation, the removal of lactic acid is much more rapid than the resynthesis of glycogen (6, 12). In man, Margaria and associates (14) have shown that at most one-tenth of the lactic acid formed during exercise is burned; that up to a considerable rate of work no extra lactate appears in the blood; and that in spite of this, some extra oxygen is consumed in recovery. They called this the alactacid oxygen debt, and concluded that when the excess

oxygen consumption is less than 3 liters, it is not employed in burning lactate. Under the special conditions of our experiments, however, about the same extra oxygen consumption is found, but with a remarkable rise in blood lactate. As shown above, the speed of removal of lactate does not change when all the extra oxygen has been used. All these facts suggest that the removal of lactate is at least partly independent of the extra oxygen used; in other words, that oxygen is not used necessarily in the removal of lactate from the blood stream.

V. *Lactate diffusion from the muscles to the local and general circulation.* In dealing with the dynamics of lactate diffusion from muscle to circulation, we have to consider three fundamental factors: *a*, the absolute amount of lactate accumulated in the muscles; *b*, the speed of the circulation through the tissues where the lactate is accumulated; *c*, the permeability of the muscle cell membrane to lactate.

In order to find out how the lactate produced in the muscles diffuses into the local circulation, the following experiment was employed:

The subject lay on a bed and the circulation of both arms was cut off with sphygmomanometer cuffs at the level of the distal insertion of the deltoids. Then the subject performed exercise with both hands by squeezing rubber bulbs rhythmically at a rate of 60 squeezes per minute. After some time, usually 1 minute 25 seconds, the pressure and the exercise were stopped at the same time and the subject remained resting in bed for 10 minutes. Venous blood was drawn at intervals from an antecubital vein throughout the work and rest periods. The results of some of these experiments are assembled in figure 3. The first part shows how the lactate reaches its maximal value in the local venous blood and the second, the manner in which it decreases.

The data suggest the following interpretations: *A*. At the simultaneous cessation of exercise and ischemia lactate diffuses into the blood stream and decreases promptly after it reaches its maximal concentration. *B*. The shape of the curve in its rise and the early abrupt drop of lactate concentration in local venous blood indicate: *a*, its high diffusibility; *b*, that its production ceases as soon as exercise ceases.

Our results are in many respects similar to those obtained by Hill and collaborators (10) in their experiments "in vitro" with a thin strip of muscle, and with those of Eggleton and Evans (4). Our experiments cannot be compared with those of Flock and Bollman (6) or with those of Sacks and Sacks (19) in which a short period of anaerobiosis in muscles contracting with intact circulation ends with increase of blood flow. In ours, the anaerobiosis is present throughout the whole period of obstruction of the circulation, and the production of lactic acid instead of ceasing after a few seconds of exercise, continues, and is a function of three factors: *a*, muscular mass; *b*, intensity of exercise performed, and *c*, time of arterial obstruction.

## SUMMARY

1. Certain interrelations between oxygen consumption, lactic acid production and removal from the blood stream have been studied on normal subjects whose limbs were rendered ischemic while they were walking in a steady state.

2. During the ischemia, ventilation and oxygen consumption were reduced by about 25 per cent.

3. After the ischemia was relieved, ventilation, oxygen consumption and blood lactate all increased far above the control value, and then returned to this level.

4. The process of restoration was much more rapid if the subject continued to walk than if he began to rest when the ischemia was relieved.

5. The amount of extra oxygen used per gram of lactate removed from the body was far greater at rest than during work.

6. No simple relation was found between the rate of repaying the oxygen debt and the rate of removal of lactate. The rate of removal of lactate was the same after the oxygen debt was paid as it was at the beginning of the recovery period.

7. Diffusion of lactic acid from the working muscles to the local venous blood was a rapid process.

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# THE INFLUENCE OF RICKETS AND OF THE HEALING OF RICKETS ON THE MECHANICAL PROPERTIES OF THE TIBIAE OF RATS[1]

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It is the purpose of this paper to present some results obtained in the study of the influence of a healing diet on the mechanical properties of bones that had been rendered rachitic and then healed for varying periods. Despite the extensive studies on rickets, there is little information about the subsequent influence of rickets on bones after healing. It has been tacitly assumed, once roentgenological healing of rickets could be demonstrated, together with restoration of calcemia, phosphatemia and blood phosphatase, that rickets had left the organism in an optimal physiological state except for any gross skeletal deformities which might remain. Such a conception of rickets is probably too restricted. There is abundant evidence in the literature to support the thesis that rickets is a constitutional disease. Hess (1) has stated that "rickets is a constitutional, metabolic disorder and not simply a derangement of the histological features of the epiphyses. However, it suggests, as a corollary, that there may be a form of rickets associated with but little deformity—a type quite as significant from a metabolic standpoint as that which is characterized by enlargement of the epiphyses, rosary and bony malformation." According to this view, it is possible that the same pathological processes which can cause bony malformations may also initiate subtle and minute pathology in other organs. The recent literature in the field of rickets tends to substantiate this idea.

Therefore, it is entirely conceivable that rickets, clinical or subclinical, might leave residua upon which disease entities are built later in life. The evidence for such a conception has been lacking in the past because of the difficulty of showing objectively that a given disease occurring in adult life could be influenced by or even be a direct result of rickets in childhood. A step in this direction was made by Clark and Mrgudich (2), who investigated the internal structure of bone by means of x-ray diffraction patterns. They observed that healed rachitic bone had lost the preferred longitudinal orientation of the inorganic crystal micelles. This was the first tangible evidence that the healed rachitic bone might be permanently altered, and it raises the question whether such bone can be as efficient mechanically as normal bone. It was in an attempt to answer this question that the present investigations were begun.

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[2] Submitted as a thesis in partial fulfilment of the requirements for the degree of Master of Science in Physiology. Awarded Sigma Xi prize for 1942. Preliminary report presented before the American Physiological Society, Chicago, 1941.

The literature bearing on the solution of this problem is scanty. Gardner (3) has observed that estrogen-treated mice have markedly thickened bones, which suggested to him that these bones might be stronger than those of the controls. He tested this hypothesis by breaking the femurs of mice by a method similar in principle to the one used in these experiments and found that the femurs of estrogen-treated mice were stronger than those of their litter-mate controls.

EXPERIMENTAL METHODS. Albino rats were drawn from the stock colony at weaning. They were divided approximately equally into nine series, totalling 267 rats, and each series was equally subdivided into litter-mate control and experimental groups. The experimental animals were given the Steenbock-Black rachitogenic diet no. 2965 from 18 to 41 days, the controls being maintained on the stock diet of the colony (Purina Fox Chow). Severe rickets was produced in all experimental animals as evidenced by roentgenological examination.

TABLE 1

*Summary of distribution of experimental animals*

| NO. OF RATS IN EACH SERIES | AGE ON RACHITIC DIET | DAYS ON RACHITIC DIET | DAYS ON HEALING DIET | AGE KILLED | DIFFERENCE BETWEEN CONTROL AND RACHITIC BREAK, WTS. |
|---|---|---|---|---|---|
| I—21 | 25 | 20 | 65 | 110 | Not significant |
| II—30 | 34 | 41 | 42 | 117 | Significant |
| III—26 | 24 | 19 | 72 | 115 | Not significant |
| IV—37 | 24 | 18 | 275 | 317 | Not significant |
| V—35 | 23 | 37 | 42 | 102 | Significant |
| VI—33 | 24 | 20 | 72 | 116 | ♀ Significant |
| VII—30 | 24 | 20 | 65 | 109 | ♂ Significant |
| VIII—33 | 24 | 41 | 105 | 170 | Not significant |
| IX—22 | 21–24 | 38–41 | 124 | 186 | Not significant |

Differences in cross-section area between experimental and control animals were significant only for series II and V.

tion. After varying periods on the rachitogenic diet, the animals in the experimental group were given 250 units of calciferol in oil and placed on the stock diet. Prompt roentgenological healing resulted in all cases. After periods of healing ranging from 42 to 275 days, the animals were sacrificed for further study.

The animals were killed by a blow on the head and the tibiae removed, dissected free of soft tissues and broken while still green. In table 1 is shown a condensed resumé of the treatment of each series.

The apparatus used to determine the breaking weight is a modification of a standard engineering machine for testing structural materials (designed by Prof. H. F. Moore of the College of Engineering). The tibia was supported on two blunt wedges 10 mm. apart, and a blunted knife-edge was placed on the bone from above to apply pressure. The bones were always placed on the wedges in the same manner to insure uniformity. By means of a motor and suitable

reducing gear, a heavy weight was pulled slowly across the appartus so as to secure graded pressure on the bone through the knife edge. A writing point recorded the movement of the weight so that, after proper calibration, the total load on the bone at any point could be calculated. Simultaneously, a record of the deformation of the bone was obtained from a gauge graduated in 0.001 inch, attached to the knife edge. From such data, load-deflection curves could be plotted by the method of triangulation (fig. 1).

These were constructed for about half of the bones. The termination of each curve represents the breaking point. The points $YP$ and $YP_1$ represent, respectively, the yield points for each bone, when the actual elastic yield occurred. The points on the abscissa each represent one in the series of deflections, which occur on the graph at progressively shorter intervals. When these are spaced equally the curve as reproduced in the figure is then constructed.

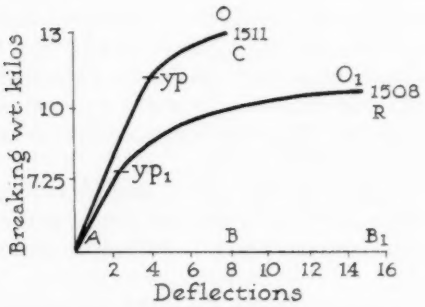


Fig. 1

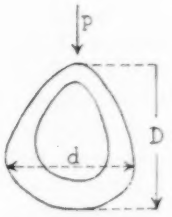


Fig. 2

Fig. 1. Load deflection curves from tibiae. C 1511. Control rat; R 1508. Series III. *YP*, point of elastic yield or permanent deformation. Areas $AOB$, $AO_1B_1$, represent amount of work done in stressing unit volume of material to point of rupture, i.e., measure of toughness of bone.

Fig. 2. Actual outline of tibiae cortex, derived as described in text.

The greater length of the curve from the rachitic bone means that more deflections occurred before breaking. The differences in the deformation of the bones in the breaking process as evidenced by the number of deflections recorded tended to be greater in those series whose healing periods were relatively short, the experimental animals showing greater deformation. The deflection values are significant only when interpreted together with their respective breaking weights.

The areas of the cross-sections of the tibiae near the point of breaking were determined in the following manner: The bones were prepared by grinding the distal fragment of the broken edge of the tibia to a plane perpendicular to its long axis as close as possible to the point of breaking. The marrow cavity was cleaned out with a sharp instrument and filled with chalk, leaving a level solid surface to which was applied a coating of black India ink. The surface chalk was then scraped out of the marrow cavity, leaving a blackened cortex which

stood out in sharp contrast to the white marrow cavity. The bones were then labeled, fixed in petri dishes with paraffin and plaster, and photographed through the bottom, together with a millimeter scale. The negatives were projected against a sheet of drawing paper, giving an enlarged image of the cortex approximately 6 cm. in diameter. The figures were outlined directly in pencil and the markings of the millimeter scale were likewise drawn in. From these figures, measurements of the depth $D$ in the direction of the load $P$ and the horizontal diameter $d$ were made for series II, III and V (fig. 2). The areas of the outlined bone cortices were taken directly from planimeter readings and the results treated statistically. The data on breaking weight and cross-section area were subjected to statistical analysis using Fischer's $t$ factor (4).

In table 2 are shown the condensed data by series on the breaking weight (with standard deviation), number of deflections during the entire time of application of the weight, and the cross section areas of the bones. Both breaking weight and cross section area were significantly lower in the experimental groups in series II, V, the females of series VI, and the males of series VII. In the other groups the trend was usually in the same direction but the differences were not great enough to be of statistical significance. However it is noteworthy that in series IX, the cross section area was greater in the experimental animals; this was true also in the females of series VIII.

The rats in series IX were on a paired-feeding regime, so that both groups were limited to the same calorie intake until one week after return to the stock diet. However, this was not done in series VIII, so it is difficult to account for these findings.

The absence of any consistent variation between the right and left bones is in agreement with the report of Weakley and Dustman (5).

The composite growth curves for series IX are omitted to conserve space but the forms of the curves are identical for both control and experimental animals. While the experimental females never reached the maximum weight of their controls, the experimental males outgrew *their* controls after about the tenth week. And yet the mean cross section area of the experimental groups of both sexes is greater than for the corresponding controls.

That rachitic bone is weaker than normal bone is an established fact. However, no literature was found regarding objective measurements of the strength of healed rachitic bone. To obtain such information has been the objective in this series of experiments.

In the interests of clarity, several of the terms used in this discussion should be defined. By "strength of bone" is meant its ability to resist force; the "elastic strength" being the bone's ability to resist force within its elastic limit; and the "ultimate strength" or "breaking strength" being its utmost ability to resist force, i.e., to resist force to the point of fracture. In this work the ultimate strength has been obtained for all the bones and is the term meant when the word "strength" is referred to. The elastic strength was determined for some of the bones in series II and V.

The fact that series II and V were on a short period of healing, whereas the other series were healed for longer periods may be the chief variable responsible

TABLE 2

*Mean values per series or group*

| SERIES | GROUP | BREAKING WT. | DEFLECTIONS 0.001" | CROSS SECTION AREA |
|---|---|---|---|---|
| | | *gms.* | | $mm^2$ |
| I | ♂C | 12,060 | 13 | 2.831 ±.0658 |
| | ♂R | 11,540 | 17 | 2.895 ±.3413 |
| | ♀C | 10,759 | 15 | 2.627 ±.1961 |
| | ♀R | 10,014 | 15 | 2.463 ±.1503 |
| II | ♂C | 13,359 ±977 | 13.6 | 2.263 |
| | ♂R | 9,345 ±1142 | 15.1 | 2.161 ±.2090 |
| | ♀C | 11,527 ±869 | 10.7 | 2.415 ±.2333 |
| | ♀R | 8,309 ±896 | 15 | 2.001 ±.2255 |
| III | ♂C | 12,951 ±846 | 14 | 2.751 ±.3303 |
| | ♂R | 12,541 ±477 | 14 | 2.621 ±.2432 |
| | ♀C | 10,283 ±1054 | 13.3 | 2.210 ±.2116 |
| | ♀R | 9,795 ±829 | 14 | 2.1497 ±.1842 |
| IV | ♂C | 16,012 ±1765 | 21 | 3.9109 ±.4342 |
| | ♂R | 15,159 ±1336 | 17 | 3.3058 ±.1406 |
| | ♀C | 12,821 ±919 | 11 | 2.7787 ±.2071 |
| | ♀R | 12,291 ±1446 | 10 | 2.7781 ±.3239 |
| V | ♂C | 14,368 ±1301 | 9 | 3.2264 ±.3923 |
| | ♂R | 10,720 ±1110 | 15.8 | 2.3555 ±.3135 |
| | ♀C | 12,539 ±1164 | 12.6 | 2.4729 ±.1968 |
| | ♀R | 9,907 ±624 | 12.6 | 2.0668 ±.2305 |
| VI | ♂C | 15,720 ±865 | 14 | 3.8336 ±.2826 |
| | ♂R | 14,937 ±1363 | 16 | 3.5142 ±.2574 |
| | ♀C | 12,871 ±1270 | 12 | 2.8852 ±.3323 |
| | ♀R | 11,500 ±1137 | 14 | 2.6709 ±.1999 |
| VII | ♂C | 14,927 ±1380 | 18 | 3.6929 ±.3071 |
| | ♂R | 12,106 ±1614 | 18.7 | 3.0199 ±.3903 |
| | ♀C | 12,478 ±1467 | 16.6 | 2.6929 ±.1965 |
| | ♀R | 11,709 ±1680 | 16.0 | 2.6516 ±.2503 |
| VIII | ♂C | 15,861 ±1896 | 7.3 | 3.7916 ±.5523 |
| | ♂R | 16,535 ±2172 | 7,0 | 3.4742 ±.5709 |
| | ♀C | 11,660 ±1089 | 9.3 | 2.6525 ±.2509 |
| | ♀R | 12,655 ±1973 | 11.0 | 2.6884 ±.3574 |
| IX | ♂C | 13,587 ±2897 | 9 | 2.4471 ±.5735 |
| | ♂R | 15,920 ±2071 | 8.4 | 3.4059 ±.6436 |
| | ♀C | 12,231 ±1452 | 7.7 | 2.4771 ±.2245 |
| | ♀R | 11,945 ±1639 | 6 | 2.5135 ±.1897 |

for the significant differences observed in these two series. Since there were no significant differences in breaking weights between the experimental and control animals in series I which had undergone healing for 65 days, the assumption

can be made that somewhere between 42 and 65 days the various factors which entered into the healing of rickets have progressed to the point where they no longer allow for differentiation of healed rachitic bones from those of their litter-mate controls by the breaking strength method herein described. With longer healing periods some of the experimental bones actually showed greater breaking strengths than those of their controls (series VIII and IX). These differences, however, were not significant.

By plotting the breaking weights (load) as ordinates and the deformations (deflection) as the abscissae, a load-deflection curve is obtained (fig. 1) from which can be read the yield point or elastic limit *YP* of the material (the point at which permanent deformity of the bone begins), which is a measure of the elastic strength of the bone. The area *AOB* under the curve is the amount of work done in stressing a unit volume of material to the point of rupture, which is a measure of the toughness of the bone. The experimental bones in series II and V subjected to such an analysis always showed a decreased elastic strength and decreased toughness when compared with similar curves made from the bones of their litter-mate controls.

The question "what are the factors which might affect the breaking strength of bone?" presents itself. On the purely mechanical side, assuming the quality of the bone to be unchanged, the cross-section area, the depth and horizontal diameter of the cross-sections, the configuration of the outlines of the cortex and the density of the bone are all to be considered. As has been mentioned, the areas and diameters have been measured but the evaluation of the configuration of the bone has not been attempted. This property must be known before the data in hand can be assembled for mathematical study to determine from mechanical laws whether the bones in these experiments have been altered in internal structure. If it can be shown that the configurations and the moment of inertia of the experimental bones have not been changed by the treatment of the rats, then the only other tenable explanation is that there has been a change in the fine structure of the bone in those series where significant changes in strength have occurred.

The cross-section areas of the cortices revealed significant differences between experimental and litter-mate controls only in series II and V, the same two series which showed significant differences in breaking strength. Similarly, the depths and horizontal diameters of the experimental bones in series II and V were significantly smaller than those of their controls.

The laws of mechanics state that a diminution in area and in the depth and horizontal diameter result in a diminution of breaking strength, assuming the quality of the material is unchanged. Hence, the bones of the experimental animals in series II and V have been altered by either: a, reducing the strength mechanically with the quality unchanged, or b, that the strength has been reduced mechanically along with some change in the quality of the bone.

In favor of the mechanical explanation is the work of Gardner (3) referred to earlier, who states that the "breaking strength was closely correlated with the amount of bone present as determined by direct observation and by density as demonstrated by x-ray photographs".

The internal structure of the bone (quality) may be altered in several ways: 1. The inorganic crystal micelles may undergo changes in orientation. 2. The inorganic crystal micelles may be altered in shape. 3. The organic matrix may be altered.

Any or all of these might affect the breaking strength of the bones. An alteration in the quality of the bone substance may be arrived at by critical analysis of the configuration of the cross-section of the cortex, but this involves an elaborate graphical and mathematical study of each individual bone from which the moment of inertia must be obtained.

It is obvious then that cross section area is not the sole factor determining the breaking stress and this is confirmed by a curve correlating breaking weight and cross section area, from which it appears that there is not a straight line correlation. There were 352 points used to determine this curve.

Still further, a curve correlating number of deflections and cross section area indicates also that there are quantitative functional factors involved in all three determinations, cross section area, breaking weight and number of deflections, since a straight line graph was not produced.

So far as this one type of test is concerned, it appears that there is no residual defect in the bones of healed rachitic rats if sufficient time is allowed. But this conclusion applies only to resistance to lateral stress. That there are *no* metabolic residua must be determined by other types of examination of bone, such as that described in the accompanying paper.

These experiments further support the growing conception of bone as a dynamically functioning organ participating in many phases of metabolism and therefore affected by many metabolic functions.

We acknowledge with gratitude the continued interest, practical suggestions and technical information supplied by Prof. H. F. Moore of the Engineering Experiment Station.

## SUMMARY

1. An apparatus for applying a quantitative breaking test to the bones of rats is described.

2. When tested by this method the tibiae of rats that have been rachitic and then healed, when compared with litter-mate controls showed significant differences in resistance to lateral breaking stress in two out of nine series of experiments involving 267 animals.

3. In one group on a healing diet for 12 weeks there were significant differences in the females, while in another on a healing diet for 9 weeks there were significant differences in the males only.

4. The metabolic significance of the data is discussed.

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# AN ATTEMPTED CORRELATION OF MECHANICAL PROPERTIES OF BONE WITH ANTIRACHITIC HEALING AND WITH MOLECULAR STRUCTURE AS DETERMINED BY X-RAY DIFFRACTION TECHNIC[1]

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Numerous studies have been made on the crystal structure of bone by x-ray diffraction technic, with the result that it is generally accepted that the pattern obtained is practically identical with those from various members of the apatite series having the general formula $3\ Ca_3(PO_4)_2 \cdot CaX_2$. Reviews of the earlier work have been given by several investigators (1, 2, 5). Taylor and Sheard (1) found that diffractograms made from powdered apatite (podolite, dahlite, or fluorapatite), dental enamel, normal bone, salivary calculus, tuberculous pulmonary calcification and synthetic tricalcium phosphate showed closely similar crystal structure.

Later workers (2) extended these observations to include a number of additional materials and confirmed the earlier observations in all essential details. In addition data were published on the lattice spacings calculated for as many as 32 lines or rings, also comparative shadow densities. It was concluded that the composition of bone may be represented by the formula $CaCO_3 \cdot n\ Ca_3(PO_4)_2$, where $n$ is not less than 2 nor greater than 3.

While these investigators did not refer to any preferred orientation of the crystals within the osseous structure and did not discuss that possibility, their figure 2, a diffractogram of dental enamel, shows clear evidence of fibering, thus indicating preferred orientation.

Cape and Kitchin (3) published a diaschema of a diffractogram of dental enamel indicating preferred coincident orientation in 9 out of 14 rings represented. The actual picture was not reproduced.

Clark, Bucher and Lorenz (4) published a single diffractogram of normal bone in which there is evidence of preferred orientation in one ring, which corresponds roughly to the position in which such orientation was later found by Clark and Mrgudich (5), although the earlier report did not discuss this feature at all.

Aside from cursory reference, little attention was given in any of these earlier papers to the possibility of altering the molecular arrangement of bone by physiological processes. Bone was regarded as a relatively fixed crystal structure mainly of heterogeneous arrangement.

J. H. Clark (6) was apparently the first to emphasize preferred orientation of patterns obtained from osseous structures, but her observations were confirmed

[1] Financial support from the Graduate School Research Board, from the Nutrition Research Laboratories and from the Ella Sachs Plotz Foundation is gratefully acknowledged.

in part by several independent investigators (5, 7, 8, 9, 10, 11). There was a progressive trend toward recognition of bone as a mobile structure responsive to physiological and pathological influences, rather than as a fixed crystal structure as first described (1, 2). Thus, Bale (12) concluded that the unit cell dimensions of bone, dentine, enamel, hydroxyiapatite and tricalcium phosphate hydrate are identical but that "present diffraction evidence is consistent with a considerable lack of homogeneity in the nature of tooth and bone substance."

There is no very satisfactory agreement on many points reported by different workers which fact has seemed to justify the further investigations reported in this paper. For example, it is not agreed as to the extent of minute alterations in chemical composition of bone under various conditions. Also Bale (12) presented dimensions of unit cell size in bone and apatite that vary considerably from those recorded by Wyckoff (13). These and other facts make it apparent then that the molecular arrangement of bone is still virgin territory and that some of the physiology of bone is dependent upon a correct comprehension of this problem.

EQUIPMENT AND METHODS. The x-ray diffraction technic has been in use for about 30 years in the study of crystal structure of metals and the molecular arrangement of rubber, textiles, plastics and other industrial materials. We could thus take advantage of a technic that has been well developed under the exacting conditions imposed by industry.

The theoretical basis for the technic has been explained by Clark (14). The phenomenon of diffraction depends on the lattice patterns in the planes of a crystal. A portion of the x-rays in a beam are diffracted so as to form a funnel-like arrangement which, on a flat film surface at right angle to the axis of the beam, will form a ring. At a constant distance the dimension of each ring will always be constant for a given substance. If the film distance and the radius of a ring are accurately determined these data may be used to calculate, by the Bragg formula, the lattice spacings in Ångström units, characteristic of each plane in a particle. These *d* values become characteristic identifications of certain planes, and a given pattern indicates a specific chemical substance.

If particles are arranged heterogeneously, or without preferred orientation, the rings will be uniform in density and width through the entire circumference. If, however, there is preferred orientation, i.e., particles are arranged in a definite way, then diffracted rays, instead of a funnel-like arrangement, will be grouped so that the ring characteristic of the particular plane involved will show fibering or arcing in that crescentic thickening will appear in one axis, and the ring will be fainter or even broken in the axis at right angle to the first.

EXPERIMENTAL MATERIAL. The skeletons of the rats used by Schiller, Struck and Reed (15) in a study of the influence of rickets and of antirachitic healing on the mechanical properties of bones had been preserved and were available for use in this study. Since all of the breaking tests had been applied to the midshaft of the tibia it was natural that we selected a contiguous area from which to grind the sections we used. This was a fortunate, if unpremeditated choice, for it was found very early that differences of considerable magnitude

exist among different bones of the same animal, and even among different parts of the same bone. Hence it is necessary only to emphasize that, unless otherwise stated, the data discussed in this paper were obtained from longitudinal slab-like sections. Each section was taken from the outer cortex of a tibia, as nearly as possible in a standard position, at the juncture of the middle and lower thirds of the shaft. At this point the shaft is of least diameter and most nearly cylindrical, and the micelles are more uniformly arranged in parallel.

Most of the bones used for diffraction studies were air dried. Sections ground from fresh bones gave diffractograms identical with those obtained from the

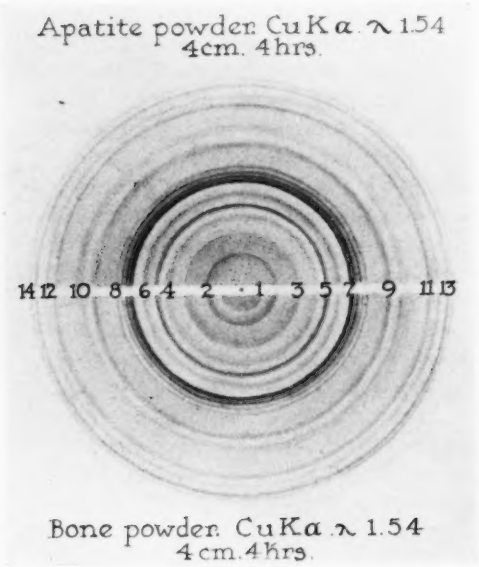


Fig. 1

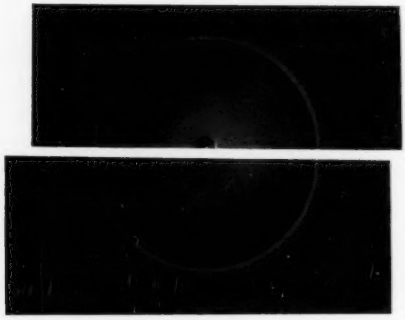

Fig. 1a

Fig. 1a. Actual positive prints of diffractograms from which figure 1 was drawn; same relative positions. Skewed arrangement due to inversion of bone powder picture to eliminate shadow of wire and bead. Many lines visible on original negative are not reproduceable in a positive print.

same material after weeks of drying. On *a priori* grounds there would appear to be no good reason to expect any alteration in crystal structure by the drying process.

Experimental results. More than 800 diffractograms have been made on bones from various sources. For purposes of analysis, patterns were obtained from powdered apatite and from powdered bone obtained by grinding the surface layer of a normal human femur. In figure 1 are semicircular reproductions of these, paired for comparison. The original films are developed as negatives and are difficult to reproduce so all of the fine detail is apparent (fig. 1a). At best, however, a drawing also leaves much to be desired. The original films from

which this figure was drawn are available for examination by anyone interested. It will be apparent, however, that except for minor variations in comparative density in some of the rings, and the presence of one additional ring in the center of the bone pattern, the two are identical. The spacings calculated from the measurements obtained from these patterns are shown in table 1. There is considerable disagreement between these data and those previously published (2, 3, 6, 8, 10, 11, 13), although in the main the discrepancies are not marked. In all subsequent references, rings will be identified by the numbers assigned in this table, and in figure 1, as well as by the *d* values.

In figure 2 are shown diffractograms of bones from animals in series IV as described by Schiller, Struck and Reed (15). That from the control animal is

TABLE 1

| RING NO. | APATITE | | | BONE POWDER | | |
|---|---|---|---|---|---|---|
| | Radius | *d* | | Radius | *d* | |
| | *mm.* | *Å.u.* | | *mm.* | *Å.u.* | |
| 1 | | | | 4.5 | 17.11 | |
| 2 | 7.5 | 8.29 | | 7.5 | 8.29 | |
| 3 | 13.0 | 4.29 | | 13.0 | 4.92 | |
| 4 | 15.75 | 4.06 | 3.89 | 15.5 | 4.12 | 3.96 |
| | 17.25 | 3.27 | | 17.0 | 3.80 | |
| 5 | 18.75 | 3.48 | 3.43 | 18.75 | 3.48 | 3.43 |
| | 19.5 | 3.38 | | 19.5 | 3.38 | |
| 6 | 21.25 | 3.20 | 3.12 | 21.5 | 3.16 | 3.12 |
| | 22.5 | 3.04 | | 22.0 | 3.08 | |
| 7 | 24.25 | 2.84 | 2.78 | 23.75 | 2.96 | 2.82 |
| | 25.5 | 2.72 | | 26.0 | 2.68 | |
| 8 | 27.5 | 2.58 | | 27.5 | 2.58 | |
| 9 | 33.0 | 2.26 | | 33.0 | 2.26 | |
| 10 | 36.0 | 2.10 | | 36.0 | 2.10 | |
| 11 | 42.0 | 1.88 | | 42.0 | 1.88 | |
| 12 | 44.5 | 1.76 | | 44.5 | 1.76 | |
| 13 | 45.5 | 1.72 | | 45.5 | 1.72 | |
| 14 | 48.0 | 1.68 | | 48.0 | 1.68 | |

Film distance 40 mm. Nos. 4, 5, 6, 7 were very broad rings, hence both internal and external circumferences were measured and the *d* values averaged.

typical of about 400 diffractograms from control animals. The most striking thing is the sharp arcing, or fibering, of no. 5 ring. The long axis of the bone is indicated by the line drawn through these arcs. Also there is evidence, less distinct, of fibering in no. 8 ring which coincides in orientation with that in no 5. The preferred orientation displayed here confirms the observations described by others (3, 5, 6, 7, 9). However, it does not confirm other aspects of the pattern described by J. H. Clark (6) in that there does not appear to be any strong orientation on an axis at right angle to that of the micelles of the bone, although there is slight widening of no. 7 opposite the gaps in no. 5.

Further, it is in complete disagreement with the pattern described by Cape and Kitchin (3) in which there was coincident fibering in 9 out of 14 rings.

The pattern, *r* in figure 2, is from an experimental animal of this same series, which was on a rachitogenic diet 18 days and on an antirachitic diet 275 days. The mechanical performance of the bones from this series (IV) as described (15) shows that, so far as the breaking test is concerned, the animals had recovered completely and the bones had regained their resistance to lateral stress.

But when examined by the diffraction technic it is apparent that the characteristic pattern of rickets is still present to a marked degree. There is definite fibering in no. 8, but that in no. 5 is much less distinct than in the control animals. And in both the orientation is in the long axis of the micelles. It must be concluded then that some observations reported previously (3, 6) were not complete. The very dark central band noted by several early workers is very faint in all of our patterns. It can only be concluded, therefore, that some of the features so prominent in many of the earlier diffractograms were due to the imperfections of equipment. At any rate the rachitic state has not produced the marked darkening of the central disk that we recently stressed (16). The

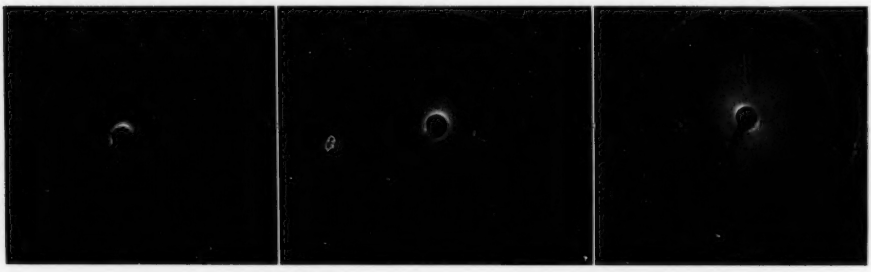

Fig. 2 Fig. 3

1762 c 1766 r

Fig. 2. 1762, Control animal. 1766, Healed rachitic animal. (See text.)
Fig. 3. Positive print from diffractogram film of spicule from buffalo skull. See text.

diffractograms reproduced in that brief report were made with the older equipment now discarded.

The rachitic state, then, produces disorientation in no. 5 ring being extended and the widest part of the shadow becoming less dense. In other bones there was complete coalescence of the arcs at one or both ends, tending to form, or actually forming, a smooth ring like that produced by powdered bone.

The fact that this condition persisted in all but 3 animals out of 37 in this series even after 275 days on an adquate diet raises a question about the significance of this change. The breaking test indicated that the bones of the rachitic animals were quite as strong as those of the control animals of this series.

The same was true of all the other series. The degree of disorientation might be expected to show an inverse proportion to the length of time on the healing diet. This has not, however, proven to be the case. If molecular orientation has any physiological significance, it must be in relation to some function not involved in resistance to lateral stress. There are several possibilities, but any discussion of them at this time would be purely speculative.

Since all of the bones used represent terminal stages of individual experiments, as all of the animals in a series were killed at one time, there is no means of knowing whether there was *any* recovery of orientation. To investigate this point an experiment was devised wherein a group of rats was rendered severely rachitic, then half were placed on an adequate diet, and the others received merely a supplement of vitamin D with the rachitogenic diet. At regular intervals one rat was taken from each of these groups and one from a control group.

At the present diffractograms indicate that reorientation to the extent of about half has occurred under the vitamin D supplement to the rachitogenic diet while the antirachitic diet has produced considerably less reorientation. This result is so bizarre that it must be repeated for confirmation. It is mentioned

TABLE 2

| RING NO. | CONTROL | | | HEALED RACHITIC | | |
|---|---|---|---|---|---|---|
| | Radius | $d$ | | Radius | $d$ | |
| | *mm.* | *Å.u.* | | *mm.* | *Å.u.* | |
| 1 | | | | | | |
| 2 | 7.5 | 8.29 | | 8.25 | 7.62 | |
| 3 | 12.0 | 5.31 | | | | |
| 4 | 17.5 | 3.76 | | 18.25 | 3.62 | |
| 5 | 19.0 | 3.50 | 3.42 | 19.5 | 3.55 | 3.40 |
| | | 3.34 | | 20.5 | 3.26 | |
| 6 | 21.75 | 3.12 | 3.10 | 21.75 | 3.12 | 3.06 |
| | 22.0 | 3.05 | | 22.75 | 3.01 | |
| 7 | 24.25 | 2.84 | 2.78 | 25.0 | 2.78 | 2.73 |
| | 25.5 | 2.72 | | 26.0 | 2.68 | |
| 8 | 27.25 | 2.60 | | 28.0 | 2.54 | |
| 9 | 32.25 | 2.37 | | 33.5 | 2.30 | |
| 10 | 38.0 | 2.08 | | 40.0 | 2.01 | |
| 11 | 41.5 | 1.92 | | 43.0 | 1.84 | |
| 12 | | | | | | |
| 13 | 46.0 | 1.71 | | 47.0 | 1.67 | |
| 14 | | | | | | |

here only to indicate the possibilities of physiological investigation by this technic.

Table 2 is constructed from the data taken from the diffractogram reproduced in figure 2. Comparison with table 1 shows that there are disagreements which may be ascribed in part to the organized gross structure of bone on a protein matrix which blots out certain rings completely while others are modified in a definite way. Since the quantitative differences are more pronounced in the healed rachitic bone, they can scarcely be ascribed to errors in measurement. It seems likely that there are, after all, some chemical differences of small magnitude.

It has been generally assumed that the orientation of crystals in a bone takes place in response to muscular activity. The only test that can be applied to this point is to examine a bone not subject to any muscular activity. After considerable searching it was found that in the supramaxillary sinus of the buf-

falo skull there are rod-like spicules of bone of the same order of dimension as a rat tibia, both ends of which are attached to the same skull bone. Therefore, it may be presumed that these have never been subject to muscular activity but only to static stress.

A diffractogram of such a bone is reproduced in figure 3. It shows all of the main rings found in the tibia, with nos. 5 and 8 oriented in exactly the same way. Incidentally, this bone had been exposed to weathering for about 60 years.

Subsequently, examination of bones unearthed in archeological excavations in Asia Minor, one of which dates to a period 3,000 years ago, the other to about 5,500 years ago, reveals characteristic orientation in both.[2]

Bearing in mind that all of the observations on rickets were made on specimens from what is probably one of the most compact bones in the rat skeleton, the cortex of the midshaft of the tibia, it is remarkable that the crystal pattern should be so mobile or so responsive to metabolic influence. It is, perhaps, not out of order to mention some data which will be reported in detail later that illustrate this point. Such influences as parathyroid, excess vitamin D, a Ca-deficient diet, strontium or magnesium substitution for calcium, and estrogens. all produce disorientation of considerable magnitude. Whether these changes are reversible has not been determined.

Clark, in a personal communication, has suggested that, instead of grinding sections, a tracer film of paraffin be applied to bones; the orientation of the latter on the micelles can then be used to determine more accurately the orientation of the micelles themselves, since paraffin gives finer fibering in a diffractogram than the bone salts themselves.

## SUMMARY AND CONCLUSIONS

1. When examined by x-ray diffraction technic, the cortices of the tibiae of albino rats show characteristic preferred orientation on the long axis of the bones, in confirmation of the reports of several earlier investigators.

2. This orientation is most pronounced in the plane having a lattice spacing of 3.43 Å.U. and corresponds to the 5th ring in the type pattern of powdered apatite.

3. The identity of bone salts with apatite is confirmed by the finding of nearly identical powder diffractograms of bone and apatite.

4. Disorientation occurs in rickets and appears to be largely irreparable.

5. Disorientation in rickets bears no direct relation to the ability of bones to resist mechanical stress since bones of rachitic rats may be restored but disorientation is not repaired completely in 275 days on an antirachitic diet.

6. The preferred orientation of normal bone is disrupted by many physiological and pathological influences, the significance of which is being investigated.

7. Whether disorientation has any physiological significance other than as an indicator of a type of metabolic disturbance cannot be determined at present.

[2] One specimen was donated by the Field Museum, through Doctor Von Bonin; the other by Professor Krogman of the University of Chicago.

Our grateful appreciation is extended to Drs. G. L. Clark and S. T. Gross, without whose assistance and advice this investigation would not have been possible; and to Dr. H. C. Struck and Mr. J. K. Ross for the care of the rat colony, thus providing a homogeneous strain of test animals.

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# THE EFFECT OF MEAT AND MEAT FRACTIONS ON THE FATTY LIVER OF THE DEPANCREATIZED AND PANCREATIC-DUCT LIGATED DOG

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Fatty infiltration of the liver has been repeatedly observed in depancreatized and pancreatic duct ligated dogs (1–4). That this was not, in the case of the diabetic animal, due to the carbohydrate disturbance was shown by the fact that adequate control of the diabetes with insulin did not prevent the fatty livers (1, 4). The condition was originally prevented by feeding raw pancreas (1, 5, 6) and in recent years by the addition of choline chloride to the diet (4, 7). Enteman and Chaikoff have reported that 35 mgm. of choline chloride per kilogram added to the diet was sufficient for the complete prevention of fatty livers (4).

In almost all studies on the production of fatty livers in diabetic dogs, the source of protein in the diets has been *whole raw lean beef*. Due to the absence of the pancreatic enzymes in depancreatized or pancreatic duct ligated dogs there is an impaired digestion and absorption of protein (2, 11). This leads to a state of protein depletion, which may be further aggravated by the increased urinary excretion of nitrogen that occurs (13). In view of the importance of protein in the prevention of dietary fatty livers (8, 10), it seemed reasonable to consider that the state of protein depletion produced in the depancreatized and pancreatic duct ligated dog might contribute to the production of the fatty livers observed. This impression was confirmed by the results obtained when dried meat powder, which is readily digested and absorbed even in the absence of the pancreatic enzymes, was substituted for raw lean beef in the diet of diabetic dogs.

This report is concerned with the effects of diets containing as a source of protein either extracted meat powder, whole raw lean beef, or meat powder plus meat extract on the fatty livers of depancreatized or pancreatic duct ligated dogs. The meat powder and meat juice were obtained from the Valentine Meat Juice Co., and we are indebted to them for the details of its preparation.[1]

The meat powder contains almost all the protein and fat of the meat. The water extractives which are removed consist of all of the water soluble constituents of muscle, including the salts, carbohydrates, inositol and various nitrogenous bases.

PROCEDURE. Four groups of dogs are reported (table 1). The first group con-

[1] The lean beef is finely ground and extracted in hot water at a temperature just below boiling point in an open steam jacketed kettle. The solution gets enough heat at this point to coagulate most of the coagulable proteins. The liquid portion is passed through a centrifuge and filter presses and concentrated in vacuo to make "meat juice." The solid residue which contains the beef protein is submitted to hydraulic pressure to get all the juice out and then re-extracted with water and finally dried in a steam dryer.

sisted of 15 dogs fed the dried meat powder. Twelve dogs were completely depancreatized and in the remaining 3 the ducts were ligated. Group II consisted of dogs fed lean raw beef; 3 were depancreatized and 3 ligated. The third group consisted of 10 dogs, 7 were given dried meat powder plus the water extractives of meat in the form of Valentine's meat juice, 15 cc. daily. (This was the amount of concentrated meat juice extracted from 300 grams of lean beef.) Six of the

TABLE 1

*Groups of dogs studied*

| NO. OF DOGS | NO. DEPANCREATIZED | NO. DUCT LIGATED | SOURCE OF PROTEIN |
|---|---|---|---|
| 15 | 12 | 3 | Dried meat powder |
| 6 | 3 | 3 | Raw beef |
| 7 | 6 | 1 | Meat powder plus meat extract |
| 3 | 3 | | Meat powder plus inositol |
| 3 | 3 | | Meat powder plus choline chloride |

TABLE 2

*Diet fed to dogs daily*

| FOOD | AMOUNT FED PER DAY | CARBOHYDRATE | PROTEIN | FAT | IODINE NUMBER OF FAT |
|---|---|---|---|---|---|
| | | Per amount fed | | | |
| | *gms.* | *gms.* | *gms.* | *gms.* | |
| Basal diet | | | | | |
| Skim milk powder | 30 | 17 | 13.0 | 0 | |
| Cracker meal | 100 | 81 | 9.6 | 4.5 | |
| Dried brewer's yeast | 10 | | | | |
| Sugar | 30 | 30 | | | |
| Salt mixture | 3 | | | | |
| Bone ash | 4 | | | | |
| Viosterol | 2 drop | | | | |
| Sources of protein | | | | | |
| Meat powder (Valentine) | 50 | 1.5 | 38 | 4.5 | 55 |
| or | | | | | |
| Lean beef | 200 | | 40 | 20 | 60 |
| Fat | | | | | |
| Cod liver oil | 5 | | | 5 | 167 |
| Mazola oil | 17 | | | 17 | 120 |

dogs were fed dried meat powder plus inositol, one of the water extractives of lean beef. In addition, a fourth group of 3 depancreatized dogs were given dried meat powder plus 2 grams of choline chloride daily.

The diet fed the dogs is shown in table 2. The amount of meat powder fed varied from 35 to 50 grams depending on the size of the dog. The nitrogen content of the amounts of meat powder was equivalent to that of 200 to 300 grams

of raw lean beef. The choline content of the meat powder was determined by the reineckate method of Beattie (14). The choline content of the amount of meat powder fed daily was 130 to 200 mgm. On the basis of Chaikoff's report that a dog requires 35 mgm. of added choline chloride per kilogram of body weight to prevent fatty infiltration of the liver, the dogs in this series, with the single exception of dog 15-D weighing 5 kgm., would have required from 300 to 483 mgm. daily to prevent fatty infiltration of the liver. The dog weighing 5 kgm. would have required 175 mgm. daily. The amounts of raw meat fed daily to the dogs on meat contained 150 mgm. of choline.

The dogs were kept in individual metabolism cages and fed twice daily. The depancreatized dogs received insulin daily, in amounts which permitted a mild

TABLE 3

*Liver lipids of depancreatized and duct-ligated dogs fed meat powder*

| DOG NO. | TIME AFTER PANCREATECTOMY | WEIGHT | | WEIGHT OF LIVER | TOTAL LIPIDS | TOTAL FATTY ACIDS | CHOLESTEROL | | | PHOSPHOLIPIDS | NEUTRAL FAT | AMOUNT OF MEAT POWDER FED DAILY | CHOLINE CONTAINED IN MEAT POWDER FED | ESTIMATED DAILY CHOLINE REQUIREMENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | | | | Total | Free | Ester | | | | | |
| | *weeks* | *kilo.* | *kilo.* | *gm.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *gm.* | *mgm.* | *mgm.* |
| 1-D | 6.5 | 10.3 | 8.0 | 358 | 4.4 | 3.1 | 0.27 | 0.21 | 0.06 | 2.95 | 1.17 | 35 | 135 | 360 |
| 2-D | 7.5 | 13.8 | 6.8 | 369 | 5.6 | 4.4 | 0.27 | | | 2.50 | 2.80 | 50 | 203 | 483 |
| 3-D | 10.7 | 13.3 | 12.8 | 395 | 4.5 | 3.2 | 0.23 | 0.19 | 0.04 | 2.66 | 1.34 | 35 | 135 | 465 |
| 4-D | 11 | 13.0 | 12.3 | Sampled | 4.6 | 3.4 | 0.22 | 0.19 | 0.03 | 2.57 | 1.78 | 50 | 203 | 455 |
| 5-D | 12 | 15.8 | 10.0 | Sampled | 10.3 | 9.0 | 0.23 | 0.21 | 0.02 | 1.94 | 8.10 | 35 | 135 | 553 |
| 6-L | 12 | 11.8 | 9.8 | 267 | 9.6 | 8.2 | 0.32 | 0.21 | 0.11 | 1.98 | 7.20 | 35–50 | 135–203 | 413 |
| 7-D | 13 | 8.0 | 6.4 | 232 | 12.3 | 10.4 | 0.33 | 0.21 | 0.12 | 3.29 | 8.50 | 35–50 | 135–203 | 280 |
| 8-D | 13 | 10.3 | 11.3 | 403 | 5.7 | 4.4 | 0.24 | 0.20 | 0.04 | 2.72 | 2.70 | 50–75 | 203–270 | 360 |
| 9-L | 13.2 | 13.0 | 7.4 | 212 | 11.9 | 10.5 | 0.35 | 0.22 | 0.13 | 1.89 | 9.60 | 35 | 135 | 355 |
| 10-L | 13.5 | 11.8 | 10.0 | 273 | 5.0 | 3.7 | 0.29 | 0.20 | 0.09 | 2.64 | 2.02 | 35–50 | 135–203 | 455 |
| 11-D | 19 | 10.3 | 9.5 | 480 | 7.2 | 5.8 | 0.24 | 0.20 | 0.04 | 3.04 | 3.90 | 50 | 203 | 360 |
| 12-D | 22 | 10.3 | 7.8 | 521 | 10.8 | 9.4 | 0.24 | | | 2.12 | 8.40 | 50 | 203 | 360 |
| 13-D | 26 | 11.3 | 9.2 | 425 | 7.9 | 6.5 | 0.22 | | | 3.00 | 4.70 | 50 | 203 | 395 |
| 14-D | 33 | 9.0 | 9.2 | 390 | 13.1 | 11.5 | 0.27 | | | 2.04 | 10.80 | 50 | 203 | 315 |
| 15-D | 43.4 | 4.5 | 5.3 | 283 | 5.8 | 4.6 | 0.21 | | | 2.58 | 3.10 | 50 | 203 | 175 |

glycosuria. Urine analyses were done daily and blood sugars at weekly intervals. Liver lipids were done by the methods previously described from this laboratory (15).

In ligating the pancreatic ducts two procedures were used. In dogs 6-L and 10-L in group I, in 16-L in group II, and in 23-L in group III, the ducts were completely ligated and the portion of the pancreas adherent to the gut and containing the ducts was stripped off and removed. The exposed ends of the remaining pieces of pancreas were covered with peritoneum. There was no connection therefore between the portions of pancreas left and the gut. These animals were, of course, partially depancreatized, but none of them developed diabetes. In the other ligated dogs no portion of the pancreas was removed. After ligation

of the ducts, a piece of omentum was inserted between the ducts and the gut to prevent the re-establishment of any connection.

RESULTS. *The results on the 15 dogs fed meat powder are shown in table 3.* The dogs were observed for periods varying from 6.5 to 43 weeks following operation. In 9 dogs the total liver lipids were below 8 grams per cent. In the other 6, the total lipids varied from 9.6 to 13 grams per cent. The other lipid fractions were within normal limits, except for the increase in fatty acids and neutral fat that occurred when the total lipids were elevated. *No fatty infiltration of the liver was observed in the dogs on meat powder alone up to intervals of 11 weeks following operation.* In 2 dogs after 12 weeks the total liver lipids were 10 and 9.6 grams per cent. In 4 dogs after 13 weeks the liver lipids were quite normal in 2 (5 grams per cent) and in the other 2 there was an increase to 12 grams per cent. Five dogs were observed for periods varying from 19 to 43 weeks. The liver lipids

TABLE 4

*Liver lipids of depancreatized and duct-ligated dogs fed raw beef*

| DOG NO. | MEAT FED | TIME AFTER PANCREATECTOMY OR LIGATION | WEIGHT | | WEIGHT OF LIVER | TOTAL LIPIDS | TOTAL FATTY ACIDS | CHOLESTEROL | | | PHOSPHOLIPIDS | NEUTRAL FAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | | | | Total | Free | Ester | | |
| | *gm.* | *weeks* | *kilo.* | *kilo.* | *gm.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 16-L | 200 | 8 | 11.8 | 6.8 | 370 | 24.2 | 21.1 | 0.22 | | | 2.44 | 20.5 |
| 4-D* | 200 | 13.6 | 12.3 | 12.3 | 610 | 14.7 | 13.1 | 0.44 | 0.17 | 0.260 | 1.76 | 12.3 |
| 17-L | 150 | 14 | 17.8 | 13.5 | 660 | 29.9 | 25.9 | 0.28 | 0.15 | 0.135 | 2.32 | 25.5 |
| 18-L† | 150 | 15.3 | 18.3 | 13.0 | 385 | 20.6 | 17.6 | 0.30 | 0.19 | 0.112 | 1.47 | 17.0 |
| 19-D | 200 | 21.4 | 11.0 | 12.0 | 471 | 28.8 | 27.2 | 0.31 | | | 2.52 | 26.0 |
| 20-D | 150 | 33 | 23.8 | 10.0 | 734 | 32.0 | 26.9 | 0.30 | 0.19 | 0.113 | 1.70 | 20.2 |

* Dog given meat powder for 11 weeks. Liver biopsied and analyzed (table 1). Dog then changed to whole meat which she received for 14 weeks.

† Pancreatic ducts ligated and separated from duodenum by omentum.

were normal in 3, and had risen to 10.8 and 13 grams per cent in the other 2. The dog that was observed for 43 weeks had normal liver lipids, 5.8 grams per cent.

The findings in the dogs on meat powder were particularly striking when compared to the results of feeding raw beef. Of 6 dogs (table 4) on raw beef, all developed fatty infiltrations of the liver. The lowest degree was 14.7 grams per cent after 15 weeks, and 4 of the other dogs had more than 24 grams of fat per 100 grams of liver. Dog 4 was particularly interesting because prior to receiving raw lean beef she was fed dried meat powder for 11 weeks. At the end of this time, the liver was sampled and on analysis the total liver lipids were 4.59 grams per cent (table 3). The diet was then changed to raw beef and within 15 weeks the total lipid had risen to 14.7 grams per cent. The other lipid fractions in the livers of the dogs on raw meat showed a sharp increase in neutral fat and fatty acids.

Naturally the results with dried meat powder, contrasted with the results on raw beef, suggested that some component of the extractive fraction of meat was

effective in increasing the deposition of fat in the liver. Therefore, depancreatized and duct ligated dogs were fed 50 grams of meat powder plus 15 cc. of the meat extract. The results are reported in table 5. In 2 of the dogs (nos. 24 and 27) the meat extract was increased to 30 cc. The results obtained with this combination did not result in an increase in the total liver lipids in all the dogs. Three of the dogs (21, 22, 23) developed a pronounced degree of fatty infiltration, and this occurred after intervals of only 5, 7 and 9 weeks following operation. In

TABLE 5

*Liver lipids of depancreatized dogs fed meat powder plus meat extract or inositol*

| DOG NO. | TIME AFTER PANCREATECTOMY | WEIGHT | | WEIGHT OF LIVER | TOTAL LIPIDS | TOTAL FATTY ACIDS | CHOLESTEROL | | | PHOSPHOLIPIDS | NEUTRAL FAT | AMOUNT OF INOSITOL FED DAILY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | | | | Total | Free | Ester | | | |
| | | Dogs on meat powder and meat extract | | | | | | | | | | |
| | *weeks* | *kilo.* | *kilo.* | *gm.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *gm.* |
| 21-D | 5 | 9.8 | 6.8 | 377 | 21.4 | 19.5 | 0.25 | 0.19 | 0.06 | 1.92 | 19.2 | |
| 22-D | 7 | 9.8 | 7.0 | 456 | 20.2 | 18.3 | 0.29 | 0.18 | 0.09 | 2.05 | 17.8 | |
| 23-L | 9 | 9.3 | 6.8 | 294 | 21.5 | 19.8 | 0.23 | 0.15 | 0.08 | 1.51 | 19.7 | |
| 24-D | 12 | 12.5 | 8.3 | 416 | 12.50 | 11.1 | 0.21 | | | 2.04 | 10.2 | |
| 25-D | 21.5 | 10.0 | 11.0 | 448 | 3.94 | 2.8 | 0.21 | | | 2.73 | 1.0 | |
| 26-D | 24 | 11.8 | 9.3 | 550 | 7.96 | 6.6 | 0.22 | 0.20 | 0.02 | 2.73 | 5.0 | |
| 27-D | 44 | 9.8 | 11.0 | 450 | 5.06 | 3.9 | 0.24 | | | 2.08 | 2.8 | |
| | | Dogs on meat powder plus inositol | | | | | | | | | | |
| 28-D | 23 | 12.0 | 12.8 | 504 | 6.2 | 4.84 | 0.21 | 0.19 | 0.02 | 2.97 | 3.0 | 1 |
| 29-D | 9.5 | 9.8 | 7.3 | 457 | 26.6 | 24.6 | 0.25 | 0.18 | 0.07 | 1.67 | 24.6 | 2 |
| 30-D | 19 | 7.8 | 6.5 | 298 | 19.7 | 18.0 | 0.26 | | | 1.98 | 17.5 | 2 |

TABLE 6

*Effect of choline chloride, 2 grams daily, on the liver lipids of depancreatized dogs fed dried meat powder*

| DOG NO. | TIME AFTER PANCREATECTOMY | WEIGHT | | WEIGHT OF LIVER | TOTAL LIPIDS | TOTAL FATTY ACIDS | CHOLESTEROL | | | PHOSPHOLIPIDS | NEUTRAL FAT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | | | | Total | Free | Ester | | |
| | *weeks* | *kilo.* | *kilo.* | *gm.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 31-D | 11 | 10 | 7.8 | 371 | 5.3 | 2.54 | 0.246 | 0.205 | 0.041 | 2.9 | 0.60 |
| 32-D | 19 | 9.3 | 4.8 | 213 | 9.4 | 5.8 | 0.296 | 0.258 | 0.038 | 4.5 | 2.90 |
| 33-D | 26 | 12.8 | 11.8 | 406 | 7.9 | 4.1 | 0.268 | 0.242 | 0.026 | 3.5 | 1.85 |

the dogs on meat powder alone, no increase in liver fat occurred until an interval of at least 12 weeks following operation had elapsed. Dog 24 on meat extract, was sacrificed 12 weeks after depancreatization; the liver lipids were 12.5 grams per cent. The other 3 dogs were examined after longer intervals (22–24 weeks) and the liver lipids were normal. The question arose as to whether the lipotropic effect of meat powder might not have succeeded in counterbalancing the effect of the meat extractives as time went on.

Because of the fact that inositol is present in meat extract, 3 dogs were given meat powder plus inositol. *When only 1 gram of inositol was fed daily* for a period of 23 weeks, there was no increase in the total liver lipids. However, *when the dose was increased to 2 grams daily*, 2 dogs developed marked fatty infiltration of the liver, after periods varying from 9.5 to 19 weeks following pancreatectomy.

As the amount of choline in the meat powder was less than would be required to prevent fatty infiltration of the liver, 3 depancreatized dogs were given dried meat powder plus 2 grams of choline chloride daily (table 6). They were kept on this for periods of 11, 19 and 26 weeks which intervals corresponded to the intervals that dogs were kept on meat powder alone. In dogs on meat powder alone, the liver lipids after these intervals were 4.6, 7.2 and 7.9 grams per cent. In the dogs on meat powder plus choline chloride, the liver lipids were 5.3, 9.4 and 7.9 grams per cent. The addition of choline to the meat powder did not influence the total liver lipids.

Discussion. In the depancreatized and duct ligated dogs fed raw beef severe fatty infiltration of the liver occurred within 8 weeks after operation and the increase in liver fat was present throughout the experimental period, as shown by analyses done after 8, 14, 15, 21 and 33 weeks. The livers of the dogs fed dried meat powder and analyzed at corresponding intervals showed no comparable fatty infiltration. In the livers of the dogs fed dried meat powder plus all the extractives there occurred an initial increase in fatty infiltration which did not persist beyond the 15th week. However, when inositol, one of the extractives, was fed there was a marked increase in liver fat after intervals of 9 and 19 weeks. The fact that fatty infiltration of the liver did not persist in the dogs fed the meat powder plus all the extractives suggests that the lipotropic effect of the meat powder counteracted the effect of the extractives when fed in the form of meat juice. These results demonstrate the lipotropic effect of meat powder and conversely the "lipogenic" effect of raw meat in dogs deprived of the external pancreatic secretion. The experiments also indicate that the effect of raw meat is due to some substance or substances contained in the extractives of meat. As regards the fat promoting possibilities of the water extractives of meat, Marks and Young (16) observed that in dogs rendered permanently diabetic by injections of an extract of the anterior pituitary gland, ketosis did not occur if the animals were fed meat which had been extracted with hot water. When raw whole meat was fed, ketosis developed. The investigators mention that "the promoter of ketogenesis is not protein per se, but some water soluble constituent of muscle tissue." They do not mention the condition of the livers of the animals but it is interesting that in their dogs raw meat led to an increased excretion of ketone bodies which suggests that the rate of production of ketone bodies by the liver was increased.

The administration of choline has been one of the methods of preventing fatty infiltration of the liver. In depancreatized dogs 35 mgm. of choline chloride per kgm. added to the diet daily was sufficient to prevent fatty livers. As we have pointed out, the amounts of choline contained in the meat powder fed to the dogs in this study would not have been sufficient to prevent the deposition of

fat in the liver (table 3). Furthermore, *the choline was not added to the diet in the form of choline chloride* but was only that which the dried meat powder contained. This is important because raw lean beef also contains choline, about 75 mgm. per 100 grams. The dogs on raw lean beef (table 4) received about 150 mgm. of choline in the 200 grams of beef fed, yet all these dogs developed fatty livers. Depancreatized dogs reported by Chaikoff received 500 grams of lean beef daily, which would contain 340 mgm. choline, and yet *unless choline* was added as such the animals all developed fatty livers. The absence of any appreciable degree of fat deposition in the livers of depancreatized dogs fed dried meat powder suggests that the lipotropic effect is independent of the choline content.

The question then arises as to why meat powder is effective in controlling fatty infiltration of the liver in depancreatized and duct ligated dogs. We feel that the lipotropic effect of meat powder is based on the fact that the protein body stores of depancreatized dogs become depleted. Dried meat powder is more readily absorbed and digested than raw lean beef, thus making available to the animal the protein ingested. Although excretion studies were not done in this group of dogs, the daily amount and state of the feces were observed, and the amounts were not increased, as is the case in the depancreatized and duct ligated dog fed raw beef (2). The fatty liver of the depancreatized dog is therefore actually similar to the dietary fatty liver that results from low protein diets. When protein is given in a form that is more readily absorbed, despite the lack of the pancreatic juice, the state of protein depletion is counteracted and fatty infiltration of the liver does not tend to occur.

The fact that lipotropic substances such as choline prevent fatty infiltration of the liver in depancreatized dogs on raw beef diets may be due to the fact that choline stimulates the phospholipid turnover in the liver and thus counteracts the effect of the extractives of meat contained in whole raw beef. The rôle of raw pancreas, pancreatic juice, or pancreatic extracts in preventing fatty infiltration of the liver in the depancreatized dog is to increase and facilitate the digestion and absorption of the protein in the diet and thus provide the liver with an adequate amount of protein.

## SUMMARY

Twenty-six depancreatized and 7 dogs deprived of the external secretion of the pancreas were studied for periods varying from 6.5 and to 43 weeks after operation.

In 15 dogs the source of protein in the diet was dried meat powder, from which the water extractives of the meat had been removed. In 6 dogs protein was given as raw lean beef. Seven dogs received meat powder plus the water extractives of meat. Three dogs received the meat powder plus inositol, which is present in the water extractives of meat. Three dogs were given the meat powder plus choline chloride daily.

None of the 15 dogs on meat powder showed any very pronounced degree of fatty infiltration of the liver. The highest total liver lipid was 13 grams per cent. In 9 of the dogs the total liver lipids were below 7.9 grams per cent.

All of the dogs on raw meat developed pronounced fatty infiltration of the liver. This occurred within 8 weeks after operation.

Of 7 dogs fed meat powder plus all of the water extractives of meat in the form of meat juice 3 developed fatty infiltration of the liver of 20 grams per cent within 5 to 9 weeks. In another dog the liver lipids were 12.5 grams per cent after 12 weeks. The other 3 dogs were examined after 22 and 24 weeks and the liver lipids were normal. Of 3 dogs given meat powder plus inositol, the 2 that received 2 grams of inositol daily developed severe fatty infiltration of the liver.

The addition of choline chloride in dogs receiving dried meat powder did not have any influence on the total liver lipids.

It is suggested that two factors contribute to the fatty liver of the depancreatized and pancreatic duct ligated dog. First—a state of protein depletion brought about by the impaired digestion and absorption of protein occurring in such animals. Second—the presence in meat of some substance or substances which are capable of producing fatty infiltration of the liver.

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# THE ACTION OF ELECTRICAL STIMULI ON THE TURTLE'S VENTRICLE

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The observation that the latency of the ventricular responses to brief threshold electric pulses may be long (over 100 msec.) suggested that this tissue was an ideal object for the study of the events which take place during the stimulus-response delay. Knowledge of those events should give insight into the problem of the mode of action of electrical stimuli on excitable tissues.

METHOD. The turtles were pithed. The heart was then excised and a quiescent ventricle was obtained by removal of the atrium. The ventricle was laid on filter paper moist with Ringer and was fixed to a board by 3 or 4 pins. After placement of 3 to 7 electrodes the preparation was covered by a large glass funnel, thus ensuring a relatively closed moist chamber.

The electrodes were usually chlorided silver needles attached to light flexible copper wire. They were supported by plasticine blocks and adjusted so that the tips rested lightly on the external ventricular surface. Although the muscle was fixed, some movement of the electrodes was unavoidable during the ventricular contractions. This movement led to shifts of contact which resulted in artifacts in the electrical records. Thus, in figure 2, the sharp downward swing that indicates the arrival of the impulse to the proximal recording leads (see fig. 1A), is preceded by a gradual rise, which is interpreted as a movement artifact. This interpretation is based on two considerations. First, the excursion attributed to movement was always larger for the leads more distant than for those closer to the stimulated region. Second, when records were taken with the stimulating cathode as one of the leads (fig. 1C), there was never seen any initial positivity preceding the negative excursion (figs. 3, 5, 8 and 9).

The electric responses of the ventricles were sometimes recorded from a cathode-ray oscillograph, after 5 stages of direct-coupled amplification. The amplifier transmitted the electric events without distortion. More commonly, the responses were registered by means of 4 Grass ink-writing galvanometers and their associated resistance-capacity coupled amplifiers. A response could thus be recorded independently from 4 different regions in the muscle (see fig. 1A). Multiple recording, from several regions, was of advantage for the measurement of conduction velocity and for the determination of the site of origin of a stimulated impulse.

The input to the amplifiers was on push-pull. The ventricle was usually grounded through one of the fixing pins. When the responses were recorded

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at the stimulating cathode—i.e., when an electrode was used both as the cathode and as a recording lead—the ground connection was from the middle tap of a

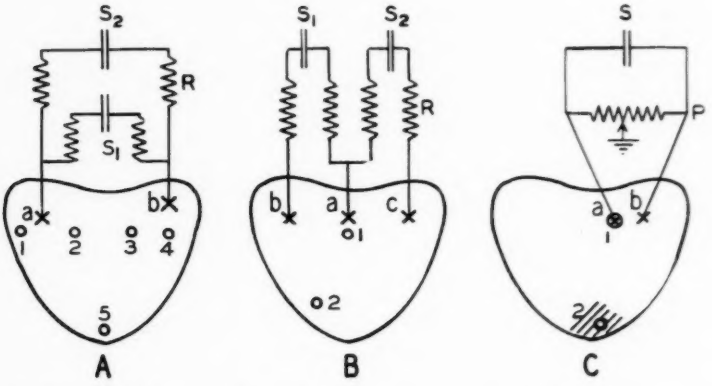


Fig. 1. Diagrams of some of the electrode arrangements used. The crosses (*a* to *c*) represent stimulating electrodes, the circles (1 to 5) leads for recording. $S_1$ and $S_2$ stand for the two brief condenser discharges. *R* denotes resistances of 10,000 to 100,000 $\omega$. *P* is a 5,000 $\omega$ potentiometer with the middle tap grounded. The hatching at the bottom of C indicates a crushed region. Other points were often crushed, e.g., the regions in contact with the stimulating electrodes *b* and *c* in B, and also those at *b* in both A and C. Additional leads were often included in arrangement B. The records in A were usually from *1*, *2*, *3* and *4*, to *5*, respectively.

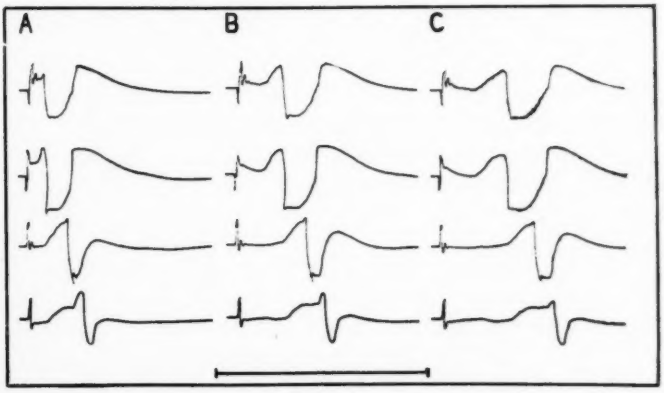


Fig. 2. Increase of stimulus-response delay shown by multiple recording. Electrode arrangement as in figure 1A. Responses to single brief stimuli. Intensity of the shocks (in conventional units): A, 35; B, 30, and C, 29. The first diphasic excursion in the records is the stimulus artifact. The beginning of the response is indicated by the sharp downward swing. The upward excursion which precedes this swing is a movement artifact. Time calibration: 1 sec. The latencies of the responses are tabulated in table 1.

This and other multiple records were taken with ink-writing galvanometers. Only the beginning of the responses is usually reproduced. The later "T" wave is omitted because unnecessary for present purposes.

5,000 ω potentiometer which shunted the stimulating electrodes (fig. 1C). Adjustment of this potentiometer minimized and balanced the stimulus artifacts.

Single records with the cathode-ray oscillograph were sometimes obtained from leads on uninjured tissue (see fig. 1B). More often, however, those records were led from an uninjured to a crushed region of the ventricle (fig. 1C). Multiple recording was usually made with all the leads on uninjured tissue (fig. 1A).

The stimuli were condenser discharges, usually with a time constant of about 0.8 msec. In 1 msec. the shocks had subsided to less than 20 per cent of the initial voltage; and in 2 msec. the discharge was for practical purposes entirely over. Two thyratron controlled stimulators were used. They were tripped by an additional condenser discharge of variable time constant. One of the stimulators was tripped directly, the other after a delay determined by a variable shunt capacity. Two independent shocks with variable time interval (1 to 2,000 msec.) could thus be delivered either through separate or through common electrodes. Resistances of 20,000 to 100,000 ω in series with the output of each stimulator ensured that independence when the two shocks were delivered to a single pair of electrodes. The condensers were always charged to 90 v. The stimuli were controlled by a lineal output potentiometer, which fractionated that voltage. Because of the series resistances the potential drop at the ventricles was much less than the output. The strength of the shocks is given as peak output volts—i.e., as conventional units for the tissue. When repetitive stimuli were applied the pulses were rectangular, with variable amplitude and duration, and with frequency controlled by an oscillator.

The stimuli were usually delivered through two electrodes placed on uninjured tissue (see fig. 1). Often, however, one of the stimulating electrodes was on a crushed region of the ventricle. The purpose of this procedure was to ensure that the impulses should arise at the undamaged region in contact with the other electrode. Tests made with the arrangement schematized in figure 1A, but with the stimulating electrode *b* on crushed tissue, indicated that the impulses always started at *a*, regardless of the polarity of the shocks.

RESULTS. A. *The stimulus-response delay.* The latency of a response recorded at some distance from the stimulated region depends on the duration of three different processes: the utilization period of the stimulus, the stimulus-response delay at the site of origin of the impulse, and the conduction time from this site to the recording point.

The utilization time can be made negligibly small by using sufficiently brief shocks. This is especially true in the heart, where the other time intervals are long. The duration of the shocks used in the present experiments (time constant 0.8 msec.) may be neglected for the computation of the stimulus-response delay.

The conduction time may be eliminated by recording the responses with one lead at the site of origin. In many observations this procedure was adopted (fig. 1C). It is possible, however, that an impulse might not necessarily always start at the cathode, but in some unknown neighboring point. An undetermi-

nable time for conduction would then vitiate the measurement of the stimulus-response delay. This possibility was explored by recording the response from several leads in different regions of the muscle. The stimulating cathode was placed between two of the leads (fig. 1A). A shift of the site of origin of the responses toward either of those two leads would result in an increase of the latency at one of them, but also in a decrease of latency at the other. A lengthening of the delay of the responses, on the other hand, would cause a similar increase of the latency at both leads (fig. 2, table 1). Possible changes of conduction velocity were tested by measurements of that velocity from the records at other leads properly aligned with respect to the stimulated point.

Only rarely was there any evidence that impulses had started at points relatively distant from the cathode. In those cases the latency at one of the leads showed a sudden change which was not paralleled in the records from other regions of the ventricle. In the great majority of the experiments, however, except for the observations which will be described later under the heading "anodal stimulation," any given change of latency was approximately the same

TABLE 1

*Latencies (in msec.; error ±2) of the responses reproduced in figure 2*

The numbers 1 to 4 refer to the tracings in the figure, from above downwards; the letters A to C refer to the corresponding stimuli. The first 4 columns give the actual latencies; the columns 2–1, 3–2 and 4–3 indicate the differences of latency from one to the following recording point in the ventricle.

| | 1 | 2 | 3 | 4 | 2-1 | 3-2 | 4-3 |
|---|---|---|---|---|---|---|---|
| A | 80 | 100 | 200 | 265 | 20 | 100 | 65 |
| B | 220 | 240 | 340 | 415 | 20 | 100 | 75 |
| C | 305 | 325 | 450 | 535 | 20 | 125 | 85 |

at all the multiple leads. It is inferred, therefore, that those changes were not due to modified conduction velocity or to a shift of the point of origin of the responses, but were due to variability of the stimulus-response delay. It is inferred also that the impulses started usually precisely at the stimulating cathode.

The responses to strong or long shocks had a shorter delay than those to weak or brief stimuli. Figure 2 illustrates the influence of the voltage on the delay of responses recorded with multiple leads. The same phenomenon is illustrated in figure 3 for responses recorded at the stimulating cathode. The latencies corresponding to two series of observations with variable stimuli are plotted in figure 4, together with the conduction velocity of the impulses. Whether the strength of shocks of constant duration (A) or the duration of equally intense shocks (B) was changed, the latent period of the responses was brief and relatively constant as long as the shocks were well above threshold. As the shocks approached either threshold strength or threshold duration, the latency increased markedly.

In some ventricles just-threshold discharges were applied with various time-

constants and the corresponding voltages. Only random, but no systematic changes of delay were seen when the time-constant was systematically varied in either direction. The inference is supported, therefore, that the significant feature of the stimuli which determines the delay is the amount, in time or in strength, by which the shock exceeds threshold.

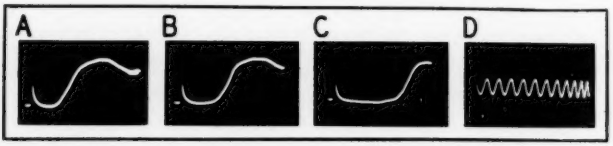


Fig. 3. Variable latency of responses recorded at the stimulating cathode. Electrode arrangement as in figure 1C. The break at the beginning of the records denotes the time of application of a cathodal stimulus. A, B and C show the responses to shocks of diminishing strength. D, time calibration, 100 cycles.

This and other records of this type were photographed from a cathode-ray oscillograph with direct-coupled amplification. Upward excursions denote negativity of the lead on intact tissue with respect to that on a crushed region.

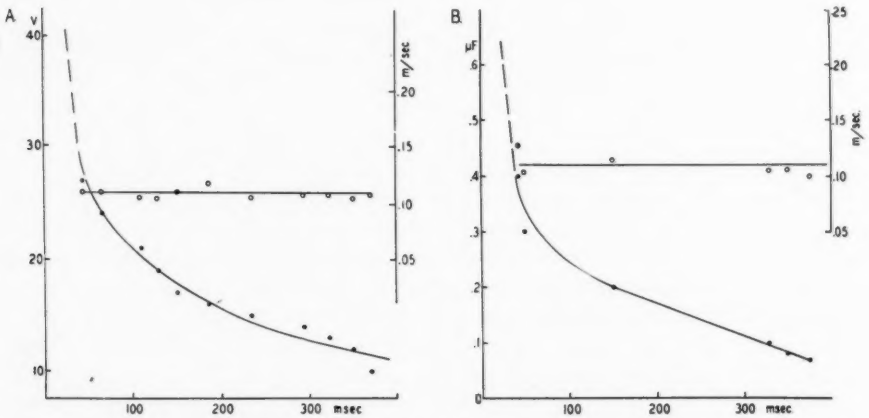


Fig. 4. Changes of latency as a function of the strength (A) or duration (B) of the stimuli. Electrode arrangement as in figure 1A. The latencies plotted (abscissae; dots; msec.) were those at lead *2* in the diagram. The conduction velocities (circles) were measured from the records from leads *3* and *4*; the conduction for each response is plotted at the abscissa corresponding to the latency of that response at *2*. The left scale of ordinates in A is the variable voltage of a condenser discharge with constant capacity (0.3 μF); the left scale in B is the variable capacity of a discharge of constant voltage (20). The right scales of ordinates are for the conduction velocities. The extrapolation of the curves at the left (broken lines) is based on the results of other similar experiments.

Pronounced systematic changes of delay could take place in the successive responses to a series of constant, slightly suprathreshold shocks delivered at a steady rate. Whether this change was an increase or a decrease depended on the rate of stimulation. Stimuli slower than 1 per 10 sec. resulted usually in a progressive decrease of latency, if the strength was such that the first shock

was just above threshold—i.e., if the delay was initially long. A progressive increase of delay, on the other hand, was usual when the shocks were applied at rates faster than 1 per 5 sec. The phenomenon is illustrated in figure 5. With these relatively fast rates, if the first shock in the series was only slightly above threshold, only a few responses were usually attainable, the heart ceasing soon to respond to the constant stimuli.

A description of the changes of excitability of the turtle ventricle after repeated activity is necessary for the understanding of the changes of delay which occur in the course of repetitive activation. In addition to the well-known excitability cycle corresponding to a single response, there are other cumulative variations of excitability which take place after repeated activity. Thus, the threshold of the tissue was usually found lower after repeated responses at a regular, relatively slow rate, so that in the course of a series progressively

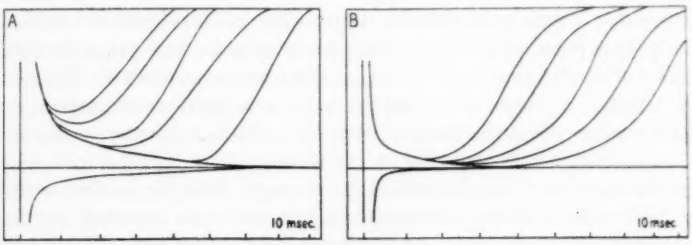


Fig. 5. Increasing latency of successive responses to constant stimuli. Electrodes as in figure 1C. The tracings were drawn from the original records by projection through an enlarger. The horizontal line toward the lower third of the figures is the resting potential line. Upward excursions denote negativity of the electrode common to the recording and stimulating circuits. The upward tracings correspond to series of cathodal shocks delivered at intervals of about 3 sec. in A and 5 sec. in B. A series of responses with increasing latency ensued; the sixth shock was ineffective. The downward excursion in both A and B corresponds to an anodal shock of the same intensity as the corresponding cathodal stimuli. In A the shocks were relatively strong; the first response has accordingly a brief latency. In B, from another ventricle, the shocks were only slightly above threshold, hence the long latencies as compared with A.

weaker shocks could cause activity. That the change was cumulative is indicated by the fact that a given weak stimulus could be effective after many responses (e.g., 10 to 15), whereas it was subthreshold when tested after only a few contractions (e.g., 4 to 6).

As opposed to the increase of excitability commonly seen in a series of responses at a slow rate, a decrease of excitability was the rule when the responses were elicited with a relatively fast frequency. Thus, in order to obtain a series of beats at 5-sec. intervals (or less), if the first stimulus was just-threshold the shocks had to be progressively intensified.

The changes of stimulus-response delay seen in a series of responses can be attributed, therefore, to changes in excitability. Or rather, in order to analogize these changes with those in figures 2, 3 and 4, the statement can again be made that, when in the course of a train of responses the constant shocks become

increasingly closer to threshold, the delay increases; and conversely, when the shocks become increasingly suprathreshold, the delay decreases.

B. *The electrical signs of the events at the stimulated region during the stimulus-response delay.* These electrical signs were studied in records obtained with the electrode arrangement shown in figure 1C. The region of the ventricle in contact with the stimulating electrode *b* was usually crushed. Shocks were delivered with the cathode at *a* (cathodal shocks) or with the anode at that electrode (anodal shocks).

Typical results are illustrated in figures 3 and 5. The part of the tracings which preceded the propagated response, when it was present, showed an asymmetry in the records obtained with cathodal, when compared to those obtained with equal anodal shocks. The negativity of the common electrode in the cathodal records usually, although not invariably, was larger and more prolonged than was the corresponding positivity in the anodal records (fig. 5). Such inequality could be due to an asymmetry in the electrodes and amplifier. To test for this possible source of error some records were taken in similar conditions but with the electrodes lying on filter paper moist with Ringer, instead of on a ventricle. These tests yielded quite symmetrical tracings. It may be inferred, therefore, that the inequality in the records from the ventricle was due to a phenomenon which occurred at the tissue.

The asymmetry was more marked for stronger than for weaker subthreshold shocks. When just-subthreshold cathodal shocks were repeated at intervals of 0.5 to 3 per sec. the residual negativity tended to decrease. There was only a slight or no difference in this negativity when a comparison was made of just-subthreshold with just-threshold shocks.

The negativity after threshold cathodal shocks gradually declined until the beginning of the propagated response. When a series of responses with progressively longer delay was obtained by repeated application of a constant shock, the negativity which preceded the spikes could be quite similar for all the records. As shown in figure 5 the responses then merely started progressively later along the curve which obtained when the shock became subthreshold. Indeetl, the responses with a long delay (over 50 msec.) could start at the resting potential level.

C. *Summation of subliminal shocks.* In a series of observations a first cathodal shock of a fixed subthreshold intensity (about 90 per cent) was followed at various time intervals by a second shock of the same polarity. The strength of the second shock which would just stimulate was measured. A summation of excitatory effects was present up to intervals of about 20 msec. (10 to 45 msec. in different preparations)—up to that time the second shock stimulated with intensities which would have been subliminal had it not been preceded by the first one. Beyond that time interval a depressed, instead of an enhanced excitability was seen. With only minor quantitative differences these results are confirmatory of those reported by Gilson and Peugnet (1932).

In another series of observations fixed brief time intervals were selected and the strength of a threshold second shock was measured when the intensity of the

first shock was varied in the subthreshold range. Typical results are shown in figure 6 for 3 time intervals in a single preparation. It is clear that even quite subthreshold shocks (20 per cent) leave a remnant of excitation for as long as 7 msec., so that the effects of a second test shock are facilitated.

Summation of two subliminal shocks could be obtained not only when the two stimuli were applied through the same pair of electrodes but also when the shocks were delivered through a common cathode and two independent anodes (fig. 1B). The results were similar with either arrangement of the electrodes.

In a third series of observations trains of repetitive stimuli, with constant brief individual duration of the pulses, were delivered through a pair of electrodes. The frequency of repetition—i.e., the interval between the pulses—was varied and the threshold intensity was measured and compared to that of a single pulse. A summation of subliminal effects was seen only when the interval separating the pulses was less than the critical interval (about 20 msec.) revealed by the two-shock experiments described above. With frequencies slower than that corresponding to this interval, no response took place unless the first shock succeeded in stimulating.

The summation with short intervals could be cumulative. Thus, a train of 20 or more pulses could be effective when a train of only 5 to 10 stimuli failed, the other characteristics of the shocks remaining constant.

D. *The interaction of two shocks of opposite polarity.* As already stated, the different latencies of the records from different points in the ventricle could indicate the site of origin of a response (p. 53). The terms cathodal and anodal will be used in this section to designate the polarity of the shocks with respect to the electrode at which responses originated.

Whether a threshold cathodal shock was preceded or followed by an anodal pulse, stimulation could be prevented—i.e., the response to the cathodal shock could be cancelled—if the interval between the two shocks and the intensity of the anodal pulse were appropriate.

In figure 7 are shown the results of a typical experiment. The cathodal shocks had a peak voltage of 110 per cent of threshold. Quite weak anodal pulses, either preceding or following the cathodal, sufficed to cancel the responses if the interval between the shocks was brief. As this interval was prolonged the anodal shocks had to be stronger for cancellation. Finally, if the interval was still longer, the strongest anodal shocks which the stimulator could deliver were incapable of preventing the responses. The results obtained from an anodal following a cathodal shock confirm similar observations made by Gilson and Peugnet (1932). The effects seen with anodal shocks preceding a cathodal stimulus extend the somewhat different test of the same problem made by these authors. In agreement with them the excitability of a stimulated region was found first decreased but later, after about 20 msec., increased after application of an anodal shock of moderate strength.

The two branches of the curve in figure 7 are not symmetrical. As is the case in that figure, the interval within which the stimulating ability of a cathodal shock could be canceled was usually briefer if the anodal shock preceded than

if it followed the cathodal. The span of this interval varied also with the strength of the cathodal shock employed. A comparison of the curves obtained first with a just-threshold, and later with a well-suprathreshold (e.g., 130 per cent) cathodal stimulus, showed that not only did stronger anodal shocks have to be used at any given interval for cancellation of the response to the greater stimulus, but that the intervals within which cancellation could be obtained were shorter.

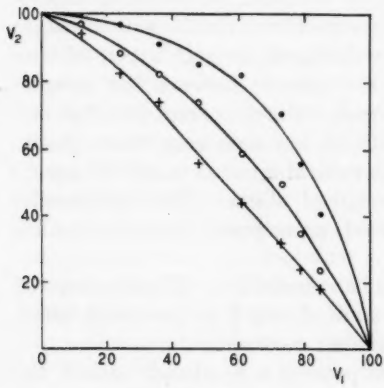


Fig. 6

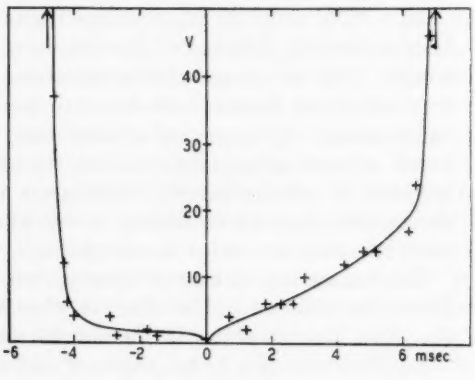


Fig. 7

Fig. 6. Summation of two subthreshold brief shocks at fixed interval. The shocks were of the same polarity and were applied through one pair of electrodes. Abscissae: intensity of 1st shock as per cent of the threshold when delivered alone. Ordinates: threshold intensity of the 2nd shock in a scale similar to that of the abscissae. Lower curve (crosses): interval between the shocks 2.2 msec. Middle curve (circles); interval 3.2 msec. Upper curve (dots): interval 7.4 msec. The observations were made on a single ventricle. The thresholds for each shock were measured at the beginning and end of each series; they remained practically constant.

Fig. 7. Cancellation of the response to a threshold cathodal stimulus by an anodal pulse. Ordinates: intensity (in conventional units) of the anodal shock sufficient for cancellation. Abscissae: interval in msec. between the two shocks; positive values indicate that the anodal shock followed, while negative values mean that it preceded the cathodal stimulus. The two arrows at the top indicate that at the corresponding intervals the strongest anodal shock delivered by the stimulator (about 80 in the scale of voltages used) failed to cancel the responses. The latency of the responses was about 30 msec. when no anodal pulses were applied.

Emphasis is placed on the fact that the critical interval beyond which a following anodal shock did not cancel the response to a preceding cathodal stimulus could be briefer than the stimulus-response delay. Indeed, when just-threshold cathodal shocks were employed the critical interval for cancellation (usually about 8 msec.) was much shorter than the delay of the response (usually about 50 msec.). This difference is strikingly illustrated in figure 8. A strong anodal shock applied 9 msec. after a cathodal stimulus not only failed to cancel the response, but actually shortened its delay.

Were the critical interval for cancellation always equal to the stimulus-response delay, then only the banal conclusion could be drawn that an anodal shock fails to stop a response after it is initiated. But, since the delay may be much longer than the cancellation interval the more interesting inference is reached that there can be a relatively long period after the application of a threshold stimulus and before the initiation of the propagated response, during which strong anodal shocks cannot prevent the appearance of the response.

In some observations a fixed brief interval (e.g., 3 msec.) was selected between the two opposite shocks. The strength of the cathodal stimulus was varied, and the voltage of the anodal shock was determined which would cancel the response. A non-lineal relationship appeared, that is, if the intensity of the

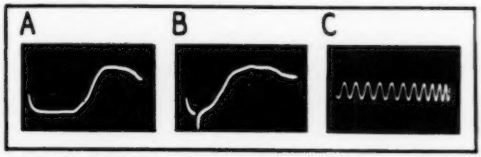


Fig. 8. Lack of cancellation of the response to a threshold cathodal stimulus by a strong anodal shock applied 9 msec. later. Electrode arrangement as in figure 1C except that the stimulating electrode *b* was on crushed tissue.

A. Response at the cathode to a threshold cathodal stimulus applied alone.

B. The same cathodal stimulus followed by an anodal shock 5 times stronger. This anodal shock did not produce any response when applied alone.

C. 100 cycles.

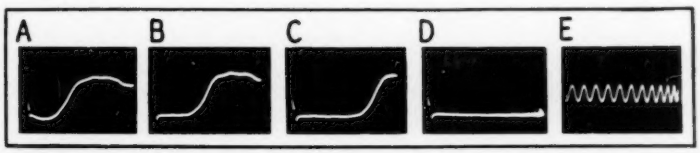


Fig. 9. Increase of delay produced by an anodal shock following a cathodal stimulus. Electrode arrangement as in figure 1C. The beginning of the records marks the time of application of the constant cathodal stimulus. The anodal shock followed after 3 msec.; its strength was gradually increased from A to D. E: 100 cycles.

cathodal shock was doubled the intensity of the anodal pulse had to be more than doubled for cancellation.

The description of interaction of two opposite shocks has dealt so far merely with cancellation of responses. When the intensity of the anodal shock was progressively increased, the interval being appropriate for cancellation, the first effect seen was a progressive lengthening of the stimulus-response delay (fig. 9). This gradual lengthening was quite similar to that which was obtained by progressively weakening a single cathodal shock (fig. 3).

All the phenomena reported in this section could be obtained not only when the two shocks were delivered through the same pair of electrodes, but also when three electrodes were employed, one of them common to the two stimuli (fig.

1B). Further aspects of the interaction of shocks of opposite polarity are described in the following section.

E. *Anodal stimulation.* By this expression is meant that invariably, when sufficiently strong shocks were applied, cardiac impulses could be initiated from the region of the heart in contact with the anode. The intensity of the shocks had to be from 1.2 to 3 times stronger than that necessary for cathodal stimulation.

These responses at the anode were not due to the "break" of the shocks—i.e., to the exponential subsidence of the condenser discharges. As is well known, the responses to the break of a current require higher intensities when the current is interrupted gradually than when it stops promptly. If in these observations the responses had been to the break of the shocks, then larger capacities, which subside gradually over a relatively long time, would have required greater voltage for anodal stimulation than that sufficient when smaller capacities were used, the discharge of which subsides promptly. The opposite, however, was observed. It is inferred, therefore, that the responses at the anode were stimulated by the "make" or onset of the pulses.

Responses at the anode to the make of a current have been often attributed to hypothetical neighboring physiological cathodes. The following observations were devised to test for the effectiveness of these assumed cathodes.

In some ventricles one of the stimulating electrodes was placed at a point closely equidistant from two recording leads. The diphasic record yielded by these leads was markedly influenced by minor shifts in the site of origin of an impulse arising between them, since the record depended on the relative time of arrival of the propagated disturbance at the two leads. Indeed, if the equidistant stimulating electrode was the cathode, shifting that electrode a fraction of a millimeter on the surface of the ventricle caused striking changes in the records. The records obtained with anodal shocks were as a rule quite similar to those which resulted from cathodal stimulation when the equidistant electrode was fixed (see the lower tracing in fig. 10). Furthermore, the responses to anodal stimuli were not changed when the anode remained fixed while the cathode was moved to different points. These results suggest that both the responses to cathodal and those to anodal stimuli arose at the point of the muscle in contact with the tip of the corresponding electrode.

In other observations the effects of a cathodal shock were studied on the response to a threshold anodal stimulus. Whether the two shocks were delivered through a single pair of electrodes, or with only one common electrode (fig. 1B), and whether the anodal stimulus preceded or followed the cathodal shock, the response could be cancelled. In other words, threshold cathodal and anodal shocks may cancel their stimulating effects even though the two shocks do not follow the same path in the ventricle. Figure 10 illustrates a typical instance.

Discussion. I. *The local response.* Katz (1937), Hodgkin (1938), and Pumphrey, Schmitt and Young (1940) have described an electric response of nerve, localized to the region of the cathode and occurring with subthreshold (as low as 50 per cent) as well as with threshold shocks. The experiments of Hodgkin

and those of Pumphrey, Schmitt and Young were similar to the observations illustrated in figure 5, that is, the records were taken from one of the stimulating electrodes and the effects of cathodal and anodal shocks were compared.

The asymmetry between the cathodal and the anodal records (p. 56, fig. 5) leads to the inference that in the heart, as in the nerves studied by these authors, there is an active, non-propagated, graded electric phenomenon elicited by electric stimuli. This phenomenon will be referred to as the local response.

Some differences between the local response of nerve and that of the ventricle may be mentioned. In nerve the local response is not seen with shocks less than 50 per cent of threshold for the propagated impulse. In the ventricle the

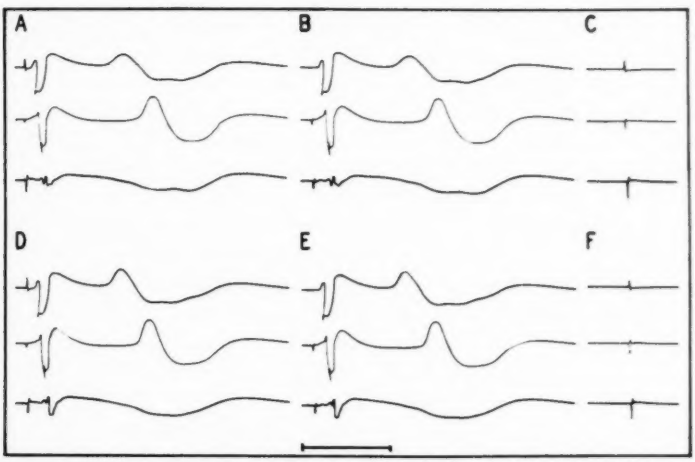


Fig. 10. Cancellation of the response to a threshold anodal shock by a threshold cathodal shock, and *vice versa*. The stimulating electrodes were placed as in the diagram in figure 1B, but the electrodes *b* and *c* were on crushed tissue. One shock (anodal) was delivered with the anode at *a* and the cathode at *b*; the other (cathodal) had the cathode at *a* and the anode at *c*. There were 4 recording leads, 3 of them referred to the 4th one.

A, anodal shock alone; B, cathodal alone; C, anodal followed (2.6 msec.) by the cathodal. D, E and F, after readjustment of the recording leads. D, anodal alone; E, cathodal alone, F, cathodal followed (2.6 msec.) by the anodal.

asymmetry between cathodal and anodal polarization records increased steadily when the shocks were strengthened. In nerve the local response can appear as a separate hump in the cathodal records; in the ventricle the cathodal tracings were approximately exponential, with no recognizable trough separating passive from active polarization (figs. 3 and 5).

II. *The stimulus-response delay.* It often has been denied that there may be a significant lapse of time between the application of a brief stimulus and the initiation of the propagated ventricular response. Thus, Blair, Wedd and Young (1941) state that the delay, if it exists at all, is a negligible part of the total latent period of a response recorded after conduction. On the basis of the present

data (figs. 2, 3 and 4) it is likely that Blair, Wedd and Young used unnecessarily strong suprathreshold shocks.

The observations described and analyzed on p. 53 (figs. 3, 4 and 5) leave no doubt that at or near threshold strength of the stimuli the delay can readily last more than 50 msec. Indeed, in preparations which had been exposed for several hours and had accordingly deteriorated, delays as long as 1 sec. were repeatably measured. The conduction velocity of these hearts was only slightly slower than when freshly excised.

The stimulus-response interval in nerve, according to Blair and Erlanger (1936), could be due to a delay of the rise of the stimulating potential because of the complicated structure of nerves as networks. Specifically, Blair and Erlanger suggest that if the shock charges a large leaky capacity which is in parallel with a resistance and the smaller capacity of the polarizable responding interface, then the maximum potential at the latter capacity would be late and could coincide with the time of response. This concept, which may be applicable to brief latencies, would require entirely improbable electrical values to explain the prolonged delays seen in the present observations.

Long ventricular latencies may be due in some instances to the initiation of impulses at some point distant from the stimulating cathode (cf. Hodgkin, 1938). The reasons have been given for dismissing this factor in the majority of the present observations (p. 53). It may be added here that, if this were the cause of increased delays, since an increase was invariably seen when the shocks were weakened from suprathreshold to just-threshold intensity, then the quite unlikely corollary would ensue that the weaker a shock, the further from the cathode the stimulated region.

III. *The intermediary process.* The fact that the stimulus-response delay can be long, leads to the inference that stimuli do not trip the spike-mechanism directly, but indirectly through an intermediary process. In the observations on nerve of Hodgkin (1938) and of Pumphrey, Schmitt and Young (1940) the spike always started at the peak of a local response. Similarly, in strips of cardiac muscle Bozler (1942) found that each spontaneous impulse was preceded by a period of slowly rising negativity, localized to the pacemaker. Such records suggest that the process responsible for the local response might be the process intermediary between the stimulus and the propagated impulse. Indeed, Pumphrey, Schmitt and Young not only proposed that the build-up of the local response to a critical level can be the tripping mechanism for the spike, but also that the important tripping factor is the local electrical potential, whether that potential be developed to the critical level by the active local response mechanism or by passive polarization due to an initial or to additional stimuli.

The present data do not support the applicability of the hypothesis of Pumphrey, Schmitt and Young to the ventricle. The responses need not begin at the peak of a rising potential; they may start at different times during the wane of the initial polarization, indeed, when that polarization has entirely subsided (figs. 3, 5 and 9). Furthermore, if the hypothesis were applicable to

the ventricle, then no matter when an anodal shock might be applied during the delay, it should be capable of canceling the response to a threshold cathodal stimulus by changing the polarization in the anodal direction. The existence of an interval in the delay period during which a response cannot be canceled (p. 58, fig. 8) opposes, therefore, the hypothesis.

The following suggestions emerge from the observations on the ventricle. The delay is due to the development of a process intermediary between the stimulus and the spike. Although the shocks elicit a local response (defined as above), this local electric phenomenon is not the intermediary process; the data do not throw any light on the possible rôle of the local response. The intermediary process does not have a significant electrical potential sign. Its rate of development to a critical tripping level is proportional to the strength of the stimulus—i.e., with strong shocks the process develops rapidly, hence a brief delay; with weak shocks it develops slowly, hence a long delay. The long delays which may ensue upon repeated stimulation (fig. 5) may be due to an influence of the relatively refractory condition of the ventricle on the rate of development of the intermediary process, or else they may indicate that the process has to build up to a higher tripping level when there is refractoriness. A subthreshold stimulus is followed by a subliminal intermediary process which is relatively long enduring, hence the possibility of summing the effects of repeated stimuli at long time intervals (p. 56). Even quite weak stimuli result in the buildup of a subliminal intermediary event, since test shocks applied later denote increased excitability (fig. 6).

Anodal stimulation is discussed below. If this action is neglected for purposes of simplification, the possibility of canceling the response to a cathodal stimulus by a following or preceding anodal shock (fig. 7) leads to the conclusion that an anodal shock opposes the development of the cathodal intermediary process. Since an anodal shock applied after a critical interval cannot cancel the response to a cathodal stimulus, it may be further inferred that the anodal influence prevents the development of the intermediary process only during the early part of that development. An alternative view which recognizes anodal stimulation would be as follows. The anodal shocks exert both a stimulating effect and an action which opposes the cathodal results. With relatively long intervals strong anodal shocks would be necessary for cancellation of the cathodal response because the intermediary process caused by the cathodal stimulus would be largely developed. Strong anodal shocks, however, would in turn elicit excitatory processes of their own which would lead to a response. Cancellation would not be obtained at long intervals, therefore, because the stimulating action of a strong anodal shock would predominate over its ability to abolish cathodal stimulation.

IV. *Anodal stimulation.* It is still an open question whether responses which arise upon the make of a current or a pulse in the neighborhood of the anode are due to anodal stimulation or are due to excitation at neighboring physiological cathodes—i.e., regions where current leaves the elements. Commenting on Rosenblueth's (1941) inference that there is anodal make stimulation in motor

nerves, Gerard (1942) states that the complicating possible action of spurious poles can hardly be dismissed on the evidence to date. We are in agreement with Gerard's statement. We would add, however, that the evidence to date does not suggest the existence of effective spurious poles, and that the burden of the proof should fall upon those who affirm their existence.

The data in section E support a direct stimulating action by the anode. If anodal stimulation were at a spurious cathode, it is difficult to understand why a second threshold cathodal shock cancels the action of the first anodal pulse when the two shocks have only one common electrode—i.e., when the currents corresponding to each of the shocks travel over largely independent intraventricular pathways (fig. 10). The second cathodal shock would be expected to sum its action with that at the initial spurious cathode, instead of opposing it. Two cathodal shocks applied with a similar arrangement of electrodes sum their effects (p. 57). The results become understandable, however, if it is assumed that the two stimulating effects occur immediately at the regions in contact with the physical common pole, and that a cathodal pulse can oppose the development of excitation by an anodal stimulus, much as an anodal pulse can oppose cathodal stimulation (p. 63).

## SUMMARY

Over 50 msec. may elapse between the time of application of a brief electric shock and the beginning of the propagated response (figs. 2 and 3). The stimulus-response delay is influenced by the characteristics of the stimuli (fig. 4); it may vary in the course of a series of responses to constant stimuli (fig. 5).

The electric phenomena which precede a response at the site of origin are described (figs. 3 and 5).

Two successive shocks interact in their effects when they have the same (p. 56, fig. 6) or the opposite polarity (figs. 7 to 10). There is a period after the application of a threshold stimulus during which a following shock of opposite polarity may cancel the response (fig. 7); there is also a later period during which the response cannot be cancelled (fig. 8).

The data suggest that the activation of the propagated impulse by an electrical stimulus is not direct, but is exerted through an intermediary process (p. 62). This process is not analogous to that which yields "local responses" in nerve (p. 62).

"Make" stimulation at the anode is described (p. 60, fig. 10) and discussed (p. 63).

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# CARDIOVASCULAR EFFECTS OF EXPERIMENTAL INSOMNIA

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Kleitman (1923) studied the physiological effect of 115 hours of experimental insomnia on the human subject, and concluded that "Respiration, heart rate, blood pressure showed a marked decrease in insomnia . . . ", attributing the decrease to the greater muscular relaxation of the sleepy subject. In two experiments on himself, the heart rates in the recumbent posture were reduced 16 and 17 per cent respectively, the systolic blood pressures 5 and 6 per cent, and the diastolics 6 and 8 per cent. Sitting, the rate was reduced only 7 per cent, while the systolic pressure increased 5 per cent and the diastolic increased 6 per cent. Of the several insomnia studies cited in a later review (Kleitman, 1929) only that of Herz (1923) included the cardio-vascular variables; in this experiment (involving a single subject) there was no reduction in the heart rate. Edwards (1941) reported that there were no significant changes in blood pressure or pulse rate as a result of the loss of 100 hours of sleep; no data were given and the criterion of significance was not mentioned.

Scott (1921) published several case reports showing substantial decrements in the Schneider (1920) cardio-vascular scores of military personnel who had had subnormal amounts of sleep. Possibly other factors were operative in producing the decrement; if not, the results are in disagreement with those obtained by Kleitman (1923) since reduction in pulse rate implies an increase in the Schneider index.

It was intimated by Kleitman that the lowered pulse rate found by him could have resulted from the tendency of the sleep-deprived subject to doze during rest while the counts were being made. In the experiment to be described, this hypothesis was tested by observation of the heart rate during one-minute standard exercises as well as at rest. The rest periods and exercises were ordered in a way that also made it possible to calculate several cardio-vascular test scores of current interest, including Schneider index scores.

METHOD AND PROCEDURE. The procedure was identical with that described by the writer in connection with another experiment (Henry, 1942). The subjects were rested in the reclining position, blood pressures were taken by auscultation, using a mercury manometer, and heart beats were recorded on a polygraph by amplified cardiac action potentials (Henry, 1937). Similar measurements were taken in the standing position, and heart beats were continously recorded during the exercise and for two and one-fourth minutes afterwards. Three exercises were given each subject using 18, 32 and approximately 33 thirteen inch stool mountings per minute. Twenty seconds after the third exercise, the subject maintained a positive intra-thoracic pressure of 20 mm. Hg as long as possible, while blowing through a Flarimeter (Wells, 1930). The tests were repeated twenty-four hours later under comparable conditions, the subject

refraining completely from sleep during the test-retest period. Eight male university students, aged 20 and 21, were tested. The experiment was conducted during the late afternoon, and no food was ingested for three hours preceding each test. The significance of the differences between experimental and control conditions was tested by conventional small-sample statistics, using Fisher's $t$ (Peters and Van Voorhis 1940). Changes in the following functional tests were also examined:

*a.* The Crampton (1913) blood ptosis test, scored from systolic pressure and heart rate changes due to changing posture from reclining to standing.

*b.* The Foster (1914) physical efficiency test, scored from standing heart rate, the rise as a result of standard exercise, and the extent of recovery in 45 seconds.

*c.* The McCurdy (1910) condition test, scored from the heart rate increase when the posture is changed from reclining to standing.

*d.* The McCurdy-Larson (1935) organic efficiency test scored from resting diastolic pulse pressures, extent of negative or positive increment in heart rate two minutes after exercise compared with the resting rate, time that 20 mm. Hg intrathoracic pressure can be maintained 20 seconds after standard exercise and vital capacity.

*e.* The pulse-ratio test (Campbell, 1925) scored from the ratio of the two minute post-exercise heart rate to the one minute resting rate.

*f.* The Schneider (1920) cardiovascular rating, scored from the items of the Crampton test with the addition of heart rate immediately after standard exercise and rapidity of return to normal.

*g.* The Tuttle (1931) physical efficiency test, scored as the amount of exercise calculated to produce a 2.5 pulse ratio by linear interpolation from two pulse-ratio tests using different amounts of exercise.

Some of these tests were modified somewhat as to the amount of exercise, as otherwise all could not have been included in a one hour test battery.

*Results at rest.* No effects were found in systolic, diastolic, or pulse pressures, either reclining or standing, or in the change in pressure due to standing. The largest $t$ was 1.50. The reclining pulse rate was 7 per cent lower under experimental conditions, a significant change since $t$ was 2.7. The standing pulse rate was 4 per cent lower, but this was not significant since $t$ was only 1.3.

*Results during exercise and recovery.* The average pulse rate during exercise was 5 per cent lower ($t = 3.0$) under experimental conditions for the first exercise, 5 per cent lower for the second ($t = 2.8$) and 6 per cent lower ($t = 3.1$) for the third. These differences are statistically reliable. During the 1st minute of recovery the rates were 7 per cent ($t = 1.4$), 9 per cent ($t = 2.4$) and 7 per cent ($t = 2.1$) lower; during the second minute of recovery they were 10 per cent ($t = 1.8$), 11 per cent ($t = 2.7$) and 5 per cent ($t = 1.0$) lower. The trend of the total recovery pulse was therefore consistent, and it was lowered by a statistically significant extent for the second exercise, but not for the first, which involved a relatively small amount of work, or for the third, where the results were complicated by the positive intra-thoracic pressure applied at 20 seconds post-exercise.

These heart-rate counts may be fractionated into fifteen second intervals (fig. 1) with very interesting results. The amount of decrease under experimental conditions becomes increasingly marked toward the end of the exercise periods and also toward the later stages of the recovery periods. There is also an increased negative phase in late recovery under experimental conditions. The differences tend to be more significant statistically during recovery from the two harder exercises (table 1).

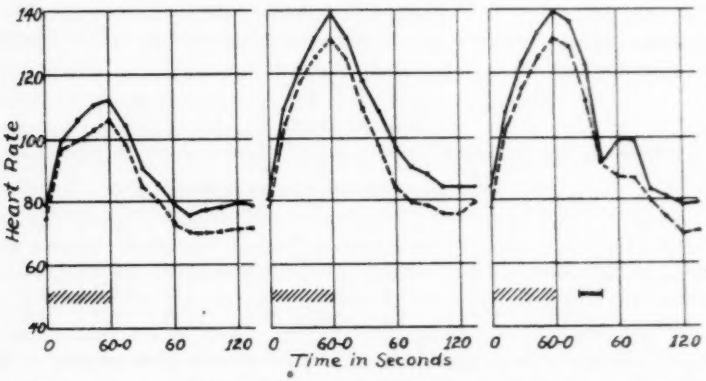


Fig. 1. Mean heart rate for 15 second periods plotted against time. Exercise periods are indicated by cross-hatching and the Flarimeter blow after the third exercise is marked by a solid bar. The broken-line curves were obtained during insomnia; the solid-line curves show the response of the same subjects under control conditions.

TABLE 1

*Fisher's t for 15 second heart-rate counts*

| NO. | DURING EXERCISE | | | | FIRST MINUTE RECOVERY | | | | SECOND MINUTE RECOVERY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0–15″ | 30″ | 45″ | 60″ | 0–15″ | 30″ | 45″ | 60″ | 75″ | 90″ | 100″ | 120″ | 135″ |
| 1 | 1.1 | 2.6 | 3.9 | 2.2 | 1.4 | 1.3 | 1.4 | 1.3 | 1.2 | 1.8 | 2.0 | 1.9 | 1.8 |
| 2 | 2.1 | 2.4 | 2.5 | 3.0 | 1.8 | 1.9 | 2.9 | 2.9 | 3.2 | 2.3 | 2.3 | 2.4 | 1.4 |
| 3 | 2.2 | 3.2 | 2.8 | 3.2 | 2.4 | 1.6 | 0.1 | 2.3 | 3.8 | 0.7 | 1.8 | 3.4 | 2.3 |

Note: Recovery in the third exercise is complicated by the Valsalva experiment. A $t$ of 2.3 is equivalent to a probability of 95/100 in favor of a real difference, which Fisher considers statistically significant (Peters and Van Voorhis, 1940, pp. 173 and 176).

The effects of the modified Valsalva experiment (i.e., the post-exercise Flarimeter blow) can be studied by examining the differences in heart rates between corresponding parts of the recovery for the second and the third exercises. These differences are shown in table 2. The Flarimeter blow began 20 seconds after the end of the third exercise, had an average duration of 23.3 seconds for the controls, and was shortened only 0.8 second under the insomnia conditions. Inspection of individual records reveals that typically there is a relative brady-

cardia[1] as the subject nears the end of his blow. Immediately after the blow there is a period of relative tachycardia lasting about 20 seconds, followed by a second period of relative bradycardia that may last a minute or more.[2] Under conditions of insomnia, the relative slowing of the heart during the blow is only about a third as great, and the onset of the second or post-blow phase of slowing is considerably delayed. There is no significant alteration in the degree of relative tachycardia or second phase bradycardia.

*Results with cardiovascular functional test scores.* These are given in table 3. It will be seen that only the Crampton test shows a decrement as a result of the insomnia and this is probably not statistically significant. The Foster and

TABLE 2

*Valsalva effect*

Mean differences between second exercise and third exercise recovery heart rates

| | SECONDS AFTER END OF EXERCISE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0–15″ | 30″ | 45″ | 60″ | 75″ | 90″ | 105″ | 120″ | 135″ |
| Control | 5.4 | 4.9 | −16.4 | 2.9 | 8.6 | −4.9 | 2.9 | −5.7 | −5.4 |
| Insomnia | 4.3 | 2.7 | −5.7 | 2.6 | 7.7 | 1.9 | −1.0 | −5.8 | −8.9 |
| *t* | 0.9 | 1.2 | 3.4 | 0.5 | 0.4 | 3.0 | 1.9 | 0.2 | 2.0 |

Note: Heart rates are in beats per minute. The flarimeter blow occurred in the 30″ and 45″ periods. A negative sign indicates a relative bradycardia.

TABLE 3

*Mean cardiovascular test scores*

| | CRAMPTON | FOSTER | MCCURDY | MCCURDY-LARSON | PULSE-RATIO | SCHNEIDER | TUTTLE |
|---|---|---|---|---|---|---|---|
| Control | 47.8 | 4.6 | 19.5 | 298.0 | −2.67 | 9.3 | 31.8 |
| Insomnia | 40.0 | 7.0 | 22.8 | 324.0 | −2.53 | 10.9 | 35.7 |
| *t* | 1.8 | 4.2 | 1.6 | 2.1 | 3.3 | 1.3 | 0.8 |

Note: A minus sign has been affixed to the mean pulse-ratio score so that a positive difference indicates improved condition of the subjects as in the other tests.

pulse-ratio tests, being heavily loaded with the recovery heart rate, show significant increments. Improvement in the McCurdy-Larson test approaches significance.

Conceivably the experimental conditions could have altered motivation, thus changing vital capacity and the toleration time for positive intra-thoracic pressure, but the changes were not significant ($t = 0.9$ and 0.5). A modified U-tube

[1] Relative bradycardia, as the term is used here, is intended to mean that the heart rate is slower in a given interval of the third exercise recovery period than in the corresponding interval of the second exercise recovery period.

[2] Records obtained in other experiments (Henry, 1942) have also been examined, and quite uniformly show that this is the usual course of the post-exercise post-blow heart rate, when the blow is maximal in length as in the present case.

Manometer Test (Warner and Hambly, 1925) using 20 mm. pressure instead of the usual 40 mm., also failed to give significant changes ($t = 1.0$).

Discussion. The positive results with reclining heart rate and negative results with blood pressures and the standing rate are explainable on the basis of Kleitman's (1923) hypothesis of "greater muscular relaxation of the sleepy subject" and a tendency on the part of the subject actually to sleep while reclining. The results during exercise and recovery cannot be explained so simply. The effects of insomnia to be accounted for are:

*a*, unaltered standing heart rate at rest;
*b*, probably unaltered heart rate during the first 15 seconds of exercise;
*c*, significantly depressed heart rate during the last 45 seconds of exercise, and during recovery from the more severe exercises;
*d*, probably increased negative phase in recovery;
*e*, lessened relative bradycardia during the Flarimeter blow;
*f*, unaltered relative tachycardia following the blow;
*g*, probably unaltered but delayed relative bradycardia following the blow.

Considering the temporal position of the alterations in rate due to insomnia, it seems possible that a lessened irritability has occurred in some part of the mechanisms responsible for the normal increase in heart rate toward the end of exercise and during recovery. It is also possible that a lessened irritability is involved in the smaller amount of relative bradycardia during the Flarimeter blow, since there is evidence that chemical factors are important in the terminal phase of the blow when it is made during recovery from exercise (Henry, 1942). It is doubtful if increased vagotone is much of a factor, as there seems to be no significant reduction in heart rate during the parts of the experiment in which the rate is known to be primarily controlled by the vagus (Dawson, 1935, pp. 254 and 487), namely, *a*, *b* and *f* above. The postulation of reduced accelerator tone (Dawson, loc. cit., also p. 243) seems to account for most of the experimental findings, although it offers no explanation for *e* or *g*. Naturally, further data are required before any theoretical explanation can be more than speculative.

It appears obvious that loss of sleep must be considered in the interpretation of functional test scores that involve pulse rate. Insomnia seems to present a special case when reduction in the pulse rate is associated with impaired, rather than improved, physical condition. This anomalous effect is greatest during exercise and the later stages of recovery, and is probably quite small under resting conditions.

Flinn (1941) observed a decrement of about 5 per cent in the pulse rate of truck drivers as a result of 0.1 to 9.9 hours of driving since the last major sleep, but was unable to offer an adequate explanation for the reduction. In the light of the present study, it seems quite possible that sleep loss may have been a causal factor in his experiment.

## SUMMARY AND CONCLUSIONS

The mean heart rate of eight male human subjects was lowered at rest in the reclining posture but not while standing, as the result of 24 hours of sleep de-

privation. It was also lowered during exercise and recovery. An increased negative phase was observed, and there was also a reduction in the amount of relative bradycardia produced by a modified Valsalva experiment performed during early recovery from exercise. A reduced irritability of some part of the mechanisms responsible for rate control during late exercise and recovery is postulated in explanation.

Scores on the Foster, pulse-ratio, and McCurdy-Larson cardiovascular tests were markedly improved as a result of the insomnia. The Schneider index was also raised, but the increase was not statistically significant.

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# DECREASED FAT APPETITE PRODUCED IN RATS BY LIGATION OF THE COMMON BILE DUCT

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In previous self-selection studies it was found that removal of most of the pancreatic tissue from rats, leaving the bile duct intact, resulted in a marked increase in the appetite for fat and a decrease in the appetite for carbohydrate. By virtue of the satisfaction of these appetites the usual diabetic symptoms, polydipsia, polyphagia, hyperglycemia, and loss of weight, did not develop (Richter and Schmidt, 1941).

It became of interest to determine whether ligation of the bile duct leaving the pancreatic tissue intact would produce any changes in appetite, especially for fat and carbohydrate.

METHODS. The experiments were performed in two series. In the first the rats had access to butter and to a mixed diet which contained a high amount of carbohydrate and no added fat. In the second series the rats had access to a full self-selection diet—11 different substances including a fat and a carbohydrate, all offered in separate containers.

In the first series the rats were kept in separate cages which contained two food cups and one graduated inverted water bottle. One food cup contained saltless butter, the other our stock diet mixture without butter. In this mixture carbohydrate constituted 58.3 per cent, and fat 1.9 per cent. Thus the rats had a choice between a fat and a diet which contained a very high amount of carbohydrate. Each rat had access also to a graduated inverted bottle filled with tap water.

Records of the intake of the butter, stock diet and water were made daily; body weight was recorded weekly. In order to obtain base lines, records were taken for several weeks before the bile duct was ligated.

In the second series the rats were placed separately in cages equipped with 3 food cups and 8 graduated inverted bottles, containing the following substances:

*In food cups.* 1. Casein (purified). 2. Dextrose, C.P. 3. Yeast (dried brewer's) or powdered whole liver.

*In graduated inverted bottles.* 4. Olive oil. 5. Cod liver oil. 6. Sodium chloride, 3 per cent. 7. Dibasic sodium phosphate, 4 per cent. 8. Potassium chloride, 1 per cent. 9. Magnesium chloride, 0.5 per cent. 10. Calcium lactate, 2.0 per cent. 11. Tap water.

From these substances normal rats make selections in such a manner that excellent growth and development are maintained.

Here again the intake of each substance was recorded daily and body weight weekly. The mineral solutions, olive oil and cod liver oil were changed twice

weekly. Records were taken for at least 20 days before the bile ducts were ligated.

At operation the bile duct was exposed through a midline abdominal incision about two centimeters long starting just below the xiphoid process. The ligatures were placed either near the duodenum, near the liver or midway between the liver and the duodenum. Since it was found (Cameron and Oakley, 1932; Richter and Benjamin, 1934) that single ligatures usually produce only a temporary obstruction, the bile duct being able to absorb the ligature and recanalize, in most instances we placed two ligatures on the duct and made a cut between them. From the results of previous experiments we knew also that after ligation the bile duct becomes distended, only mildly in some rats, very markedly in others.

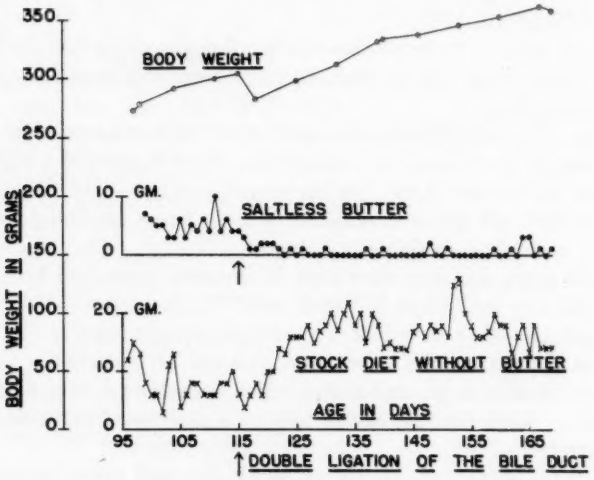


Fig. 1

In the present experiments bile ducts were either singly or doubly ligated in 25 rats. Of this number 6 died within the first 10 days with ruptured bile ducts, and their records are not included in the present paper. The mortality was greatest in the group of rats with double ligation and section near the duodenum. It was lowest in the rats with double or single ligation above the upper pancreatic duct, that is, fairly close to the liver.

At autopsy we recorded chiefly the presence or absence of distention of the duct and of bile fluid in the abdominal cavity.

Results. Figure 1 gives the record of one of the 6 rats used in the first series. This rat was placed in the experimental cage at an age of 95 days. For 10 days before operation its daily intake of saltless butter averaged 5.0 grams, and of the butterless stock diet 7.2 grams. When the animal was 115 days of age the bile duct was doubly ligated and cut near the liver. On the following day the rat ate less butter and after 7 days scarcely touched it. In marked

contrast it began to eat much more of the stock diet and after 10 days ate more than twice as much per day (16 grams) as before. At the end of 59 days, when it was killed the bile duct showed a mild degree of distention with a capacity of approximately 2 cc., but there was no jaundice.

Table 1 summarizes the results of the observations made on the 6 animals in this series. In 4 of these rats the bile ducts had been doubly ligated and cut near the liver, and in two singly ligated near the liver. Three rats with a double ligature died in 15, 24, and 25 days, respectively, and one with a single ligature died in 15 days. One rat with a double ligature near the liver was killed at the end of 59 days; another rat with a single ligature was killed after 52 days. At autopsy all of the rats showed moderate to marked distention of the bile ducts.

The table gives the average daily intake records for the last 10 days before the operation and for the last 10 days before the rat died or was killed. Usually for the first 3 to 8 days postoperatively the rats ate only small amounts of either the stock diet or of the fat. Then their appetites returned and their food-

TABLE 1

*Average daily intake for last 10 days before bile duct ligation and last 10 day period before death*

| RAT NO. | OPERATION | SURVIVAL TIME | SALTLESS BUTTER | | BUTTERLESS FOOD | | WATER | |
|---|---|---|---|---|---|---|---|---|
| | | | Before | After | Before | After | Before | After |
| | | *days* | *grams* | *grams* | *grams* | *grams* | *cc.* | *cc.* |
| 1 | Double near liver | 59 K | 5.0 | 0.2 | 7.2 | 18.1 | 20.7 | 28.0 |
| 2 | Double near liver | 25 | 3.9 | 0.7 | 10.3 | 13.6 | 26.4 | 32.6 |
| 3 | Double near liver | 24 | 4.7 | 0.3 | 5.5 | 11.1 | 14.6 | 25.6 |
| 4 | Double near liver | 15 | 4.7 | 1.0 | 7.2 | 7.5 | 20.8 | 18.9 |
| 5 | Single near liver | 15 | 4.3 | 0.6 | 8.2 | 9.1 | 18.6 | 23.7 |
| 6 | Single near liver | 52 K | 2.5 | 1.4 | 10.2 | 14.8 | 23.3 | 26.9 |
| Average..... | | 31.7 | 4.2 | 0.7 | 8.1 | 12.4 | 20.7 | 26.0 |

intake quickly reached a plateau. For the 6 rats the average daily intake of butter decreased from 4.2 grams for the last 10 days before operation to 0.7 gram for the last 10 days of life. In the same periods the intake of the stock diet with the high carbohydrate content increased from 8.1 to 12.4 grams; and water intake increased from 20.7 to 26.0 cc.

In the second series of experiments the rats had access to 11 different substances. From previous experience we know that when the vitamin B complex is offered to rats in the form of yeast or liver powder, some of the rats will take large amounts of fat and little or no carbohydrate, while others take large amounts of carbohydrate and little or no fat. In previous publications we have referred to the former as "carbohydrate burners", the latter as "fat burners". The reasons for these differences are not clearly understood but also are not relevant to the present purposes (Richter, Holt and Barelare, 1938). In the present series 7 rats were "fat burners" and 5 "carbohydrate burners" before operation. The observations on the two groups will be reported separately.

Figure 2A gives a record for one of the "fat burners". The record shows the intake of casein, dextrose, liver, and olive oil for 20 days preoperatively and until the rat died 36 days postoperatively. For the 20 days before operation the rat took large amounts of olive oil, smaller amounts of liver, and little or no casein or dextrose. On the day following double ligation and section of the bile duct near the liver the rat took no olive oil and from then on until it died it took only minimal amounts, during the last 20 days scarcely touching either olive oil or cod liver oil. In contrast it indicated a marked appetite for dextrose, which, however, did not manifest itself until quite suddenly 9 days postoperatively, when there was a sharp increase to an average of 5.9 grams per day, at which it remained until a day or two before the death of the animal. The casein appetite remained very low and the liver powder appetite decreased irregularly. The rat did not show definite changes in appetite for any of the

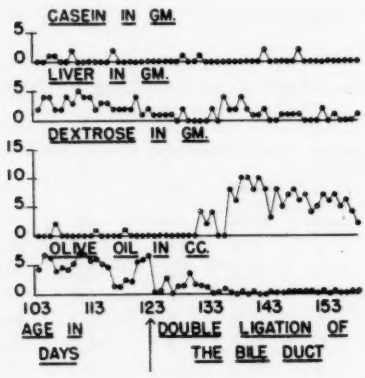


Fig. 2A

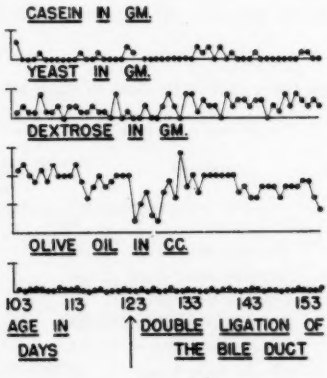


Fig. 2B

other substances. At autopsy the bile duct was very much distended, filling approximately one quarter of the abdominal cavity.

Table 2 summarizes the results of the observations made on the 7 "fat burners" and the 5 "carbohydrate burners". In 4 of the former group the bile duct was doubly ligated and cut approximately half-way between the liver and the duodenum; in one near the duodenum; in two near the liver. The survival times varied from 11 to 37 days. All 7 rats had well distended bile ducts. The table gives the average daily intake of the different substances for the last 10 days before operation and also for the last 10 day period before the rat died or was killed. The average fat intake (olive oil plus cod liver oil) decreased from 3.5 to 0.9 cc. per day after operation, with all 7 rats showing a definite decrease; the average dextrose intake increased from 2.0 to 2.9 grams, all except one rat either maintaining the preoperative level or showing an increase; the casein intake remained essentially the same while the yeast or liver intake decreased markedly in all 6 animals.

Figure 2B gives a record of one of the "carbohydrate burners". The bile

duct was ligated when the rat was 123 days of age. Postoperatively it took less dextrose for a few days, but after that continued to take high amounts up to the last day before death. It continued to take only minimal amounts of olive oil and showed a slightly increased appetite for yeast, while the casein intake remained unchanged. It was killed after 34 days. The bile duct was moderately distended, and the subcutaneous tissues showed some jaundice.

TABLE 2

*Average daily food intake for 10 days before bile duct ligation and for last 10 days before the rats died or were killed*

| RAT NO. | OPERATION | SURVIVAL TIME | OLIVE OIL PLUS COD LIVER OIL | | DEXTROSE | | CASEIN | | LIVER | | YEAST | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before | After | Before | After | Before | After | Before | After | Before | After |
| | A. "Fat burners" | | | | | | | | | | | |
| | | *days* | *cc.* | *cc.* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* |
| 7 | Double midway | 11 | 3.1 | 0.9 | 0.7 | 1.6 | 0.2 | 1.8 | 3.6 | 0.8 | | |
| 8 | Double midway | 15 | 2.4 | 0.4 | 2.4 | 2.8 | 2.8 | 1.5 | 1.6 | 0.7 | | |
| 9 | Double midway | 14 | 5.7 | 1.4 | 0.2 | 0.4 | 0.5 | 0.6 | | | 1.4 | 0.1 |
| 10 | Double near duodenum | 27 | 2.6 | 1.0 | 4.0 | 6.2 | 2.0 | 1.3 | | | 1.0 | 0.6 |
| 11 | Double near liver | 37 | 4.5 | 0.7 | 0.2 | 6.1 | 0.2 | 0.4 | 2.5 | 0.6 | | |
| 12 | Double midway | 24 | 2.3 | 1.4 | 6.7 | 3.1 | 0.4 | 0.7 | 2.1 | 0.7 | | |
| 13 | Double near liver | 11 | 3.9 | 0.6 | 0.1 | 0.2 | 0.4 | 0.7 | 2.5 | 0.1 | | |
| Average | | 19.9 | 3.5 | 0.9 | 2.0 | 2.9 | 0.9 | 1.0 | 2.5 | 0.6 | 1.2 | 0.4 |
| | B. "Carbohydrate burners" | | | | | | | | | | | |
| 14 | Double near duodenum | 17 | 0.7 | 0.5 | 8.0 | 3.3 | 2.9 | 0.6 | | | 1.8 | 0.4 |
| 15 | Double near duodenum | 34 | 0.7 | 0.8 | 10.8 | 9.7 | 0.2 | 0.0 | | | 2.3 | 2.2 |
| 16 | Double midway | 34 K | 0.6 | 0.5 | 9.2 | 9.2 | 0.4 | 0.8 | | | 1.3 | 2.4 |
| 17 | Double near duodenum | 19 | 0.7 | 0.6 | 9.1 | 7.1 | 1.1 | 0.4 | | | 1.0 | 1.2 |
| 18 | Double near liver | 24 | 0.6 | 0.7 | 7.9 | 8.6 | 3.0 | 1.6 | 1.2 | 0.8 | | |
| Average | | 25.6 | 0.7 | 0.6 | 9.0 | 7.6 | 1.5 | 0.7 | 1.2 | 0.8 | 1.6 | 1.6 |

Table 2B summarizes the results of the observations made on the 5 "carbohydrate burners". The bile ducts of three rats were doubly ligated or cut near the duodenum, in one other about midway between the duodenum and the liver, and in a fifth near the liver. The survival times varied from 17 to 34 days, at which time the last remaining rat was killed. In this group of rats which started with a very low fat intake, the fat appetite remained on the same low level after operation. With one exception all of these rats continued to eat considerable amounts of dextrose until within a few days of death. The average daily intake decreased only from 9.0 grams before operation to 7.6 grams

after operation. The fat intake of all 5 rats averaged 0.7 and 0.6 cc. respectively before and after ligation of the bile duct. The average casein intake decreased from 1.5 to 0.7 gram, with four rats showing a decrease; in three of the five rats the yeast and liver intake decreased.

The rats did not show consistent changes in appetite for any of the other substances with the possible exception of a slightly decreased appetite for sodium phosphate and magnesium chloride.

The results of these self-selection experiments are in agreement with the first series in showing that after bile duct ligation the rats did not take fat, except possibly in very small amounts, but did take carbohydrate. With only two exceptions they showed a reduced appetite also for yeast or liver powder.

Discussion. It is a well known clinical observation that the ingestion of fatty foods precipitates attacks of biliary colic in patients with bile duct obstruction. The results of the present self-selection experiments indicate that rats avoid fat even when their bile duct is occluded in such a manner that the external pancreatic secretions still are permitted to flow into the duodenum. When bile is absent the rats make selections which tend to eliminate fat from the diet, but maintain their caloric intake at an adequate level by consuming larger quantities of carbohydrates. After subtotal pancreatectomy the rats consumed large quantities of olive oil and absorbed it in the absence of a normal secretion of pancreatic enzymes. It seems likely that bile plays a predominant rôle in fat absorption, but whether it depends on a preliminary splitting of the fat by pancreatic enzymes or not is impossible to say from our experiments, since we have not been able to maintain totally pancreatectomized animals more than a very few days.

In these experiments we found another instance of the inverse relationship between the appetite for carbohydrate and fat. Thus far in all of our self-selection studies it has been found that the appetites for these two substances consistently show an inverse relationship. Thus, for example, in conditions of vitamin B deficiency rats refuse carbohydrate and eat fat. Placed on a high vitamin B diet they eat high amounts of carbohydrate and little or no fat (Richter, Holt and Barelare, 1937). In experimental diabetes produced by partial removal of the pancreas, rats refuse carbohydrate and select fat (Richter and Schmidt, 1941). Here we have seen that the rats with bile duct ligations eat little or no fat, but moderate amounts of carbohydrate.

In previous self-selection experiments we were able to show that the choices made by the rats were beneficial, that is, after various glandular or other deprivations they helped to maintain a constant internal environment, and so to prolong life or at least alleviate symptoms of disturbed metabolism. In the present experiment we have been able to demonstrate that after bile duct obstruction rats have an appetite for carbohydrate but not for fat. These choices are in agreement with clinical and experimental experience. However, we were unable to show that rats which could make their own selections did better, lived longer, etc. than those which are forced to take their food in certain fixed proportions. We did observe that some of the rats which completely refused

fat and ate only high amounts of carbohydrate had only very slightly dilated bile ducts as long as four weeks after double ligation and section; whereas, in a previous experiment in which the rats received only the stock diet with butter, we found that at the end of such periods all rats on the stock diet had markedly dilated bile ducts.

SUMMARY

1. Following ligation of the bile duct, 6 rats which were given access to butter and to a mixed diet with a low fat and a high carbohydrate content (1.9 per cent and 58.3 per cent respectively) stopped eating the butter and increased their intake of the mixed diet.

2. Following ligation of the bile duct, 11 rats which had access to casein, dextrose, olive oil, cod liver oil, 5 mineral solutions, and yeast or liver powder, ate little or no fat, but large amounts of the carbohydrate, dextrose. They ate less yeast or liver powder than before operation, and showed no definite changes in appetite for the casein or for the mineral solutions.

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# CREATINURIA IN MAN FOLLOWING THE ORAL ADMINISTRATION OF CAFFEINE[1]

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An increase in the excretion of creatine has been observed in the urine of rats (1, 2) and of rabbits (3) following the parenteral injection of caffeine. In a study of the action of this drug on the metabolic processes in man we have frequently found creatine in the urine after oral administration. These observations were but one phase of a more comprehensive study of the effect of caffeine on metabolism as shown by the respiratory exchange and the urinary constituents. Data on the respiratory exchange have been reported elsewhere (4). The purpose of the present study is to show the amount of creatine and the frequency of its appearance in the urine with different doses of caffeine and to determine whether there was any relationship between creatinuria and the respiratory exchange, carbohydrate oxidation and protein metabolism.

METHOD. Three male adults served as subjects. Each customarily used coffee in moderation but abstained from all caffeine-containing beverages for twenty-four hours before the experiment. On the morning of the experiment the subject came to the laboratory in the fasting state and reclined for 30 to 45 minutes to recover from the small amount of exercise he had taken. The bladder was then emptied and the urine discarded. The subject then reclined for a basal period of 45 minutes, at the conclusion of which the urine was collected for analysis. This collection will hereafter be designated as the "post-absorptive" urine. Caffeine alkaloid dissolved in 200 cc. water at body temperature was then taken and, in the control experiments, an equal volume of water. The amount of caffeine ingested ranged from 0.6 to 6.0 mgm. per kilo body weight. The recumbent position was resumed for one hour and forty-five minutes and at the end of this time the urine was collected for analysis. The gaseous exchange was measured throughout the post-absorptive and post-ingestion periods in the manner described previously (5).

Creatine was determined by Benedict's method (6), the picric acid used in the analysis having been purified according to his directions (7). The data obtained are taken to be true values of creatine since Beard (1) using the enzyme of Miller and Dubos has shown that the chromogenic reaction in urine is specific for creatinine.

RESULTS. The hourly urinary excretion of creatine for a period of one and three-quarter hours in all the experiments is shown in table 1. When the amount of caffeine ingested was 1.8 mgm. or more per kilo body weight, creatine appeared in the urine with but few exceptions. One milligram per kilo, however,

[1] Preliminary report: Federation Proceedings, March 16, 1942, p. 4.

induced creatinuria in only 3 out of 14 experiments. In two other experiments creatine was excreted in the post-ingestion period but these are not included in the table since it was present also in the post-absorptive urine. In none of the 8 experiments with 0.6 mgm. caffeine per kilo was there any creatine in the urine. Approximately one milligram caffeine per kilo therefore appears to be the minimal amount capable of inducing creatinuria in our subjects.

Inspection of the data in table 1 shows that there was no direct relationship between the amount of creatine excreted and the amount of caffeine ingested. The average hourly excretion was slightly less with 1.8 than with 1.0 mgm. caffeine per kilo and also less with 6.0 than with 3.0 or 3.6 mgm. The maximum excretion was approximately the same with 1.0 as with 1.8 mgm. caffeine and less with 6.0 mgm. than with 3.0 and 3.6 mgm. per kilo.

*Creatine excretion and the respiratory exchange.* Coefficients of correlation were calculated for the hourly excretion of creatine and 1, the increase in oxygen consumption above the basal level; 2, the increase in carbon dioxide elimination; 3, the total oxygen consumption; and 4, the total carbon dioxide elimination during one and three-quarter hours after the ingestion of caffeine. There was no significant correlation between the amount of creatine excreted and the respiratory exchange in terms of either the increase above the basal or of absolute amounts of oxygen consumed or carbon dioxide eliminated. The coefficients of correlation ranged from 0 to 0.10. Furthermore, there was no relationship between the occurrence of creatinuria and the height of metabolism. Since there was an increase in oxygen consumption and carbon dioxide elimination with the larger doses of caffeine, it is obviously necessary to limit comparisons of the gaseous exchange to those experiments in which the same dosage of caffeine was used. Such comparisons showed that in the different groups of experiments with various doses of caffeine, the range of increase in oxygen consumption and carbon dioxide elimination either as absolute values or as percentages above the basal was the same whether or not creatinuria occurred.

TABLE 1

*Urinary excretion of creatine after ingestion of various amounts of caffeine*

| CAFFEINE INGESTED PER KILO BODY WEIGHT | NUMBER OF EXPERIMENTS | NUMBER OF EXPERIMENTS WITH CREATINE IN THE URINE IN P-E PERIOD | RANGE | HOURLY EXCRETION OF CREATINE (AVG. OF ALL EXPTS.) | HOURLY EXCRETION OF CREATINE (AVG. OF EXPTS. IN WHICH CREATINE WAS EXCRETED) |
|---|---|---|---|---|---|
| *mgm.* | | | *mgm.* | *mgm.* | *mgm.* |
| None* | 10 | 0 | | 0 | 0 |
| 0.6 | 8 | 0 | | 0 | 0 |
| 1.0 | 14 | 3 | 0.9–1.4 | 0.4 | 1.2 |
| 1.8 | 6 | 4 | 0.6–1.5 | 0.7 | 1.0 |
| 3.0 | 12 | 11 | 0.8–4.7 | 1.6 | 1.7 |
| 3.6 | 7 | 7 | 1.5–4.2 | 2.2 | 2.2 |
| 6.0 | 10 | 8 | 0.7–2.2 | 1.0 | 1.4 |

* Control experiments.

As shown elsewhere (4) a rise in the respiratory quotient occurred after the ingestion of caffeine and reached the highest levels with the largest dosage. A critical study of the respiratory quotients of the individual experiments revealed, however, that there was no relationship between the presence of creatine in the urine and the height of the respiratory quotient. As in the case of the respiratory exchange it was necessary to limit comparisons to experiments with the same dosage of caffeine. A study of the individual non-protein respiratory quotients showed that there was no uniform or significant differences between the quotients in the experiments with and without creatinuria.

*Carbohydrate metabolism.* The presence of creatine in the urine was not related to the amount of carbohydrate oxidized. In those experiments in which creatine appeared in the urine after the ingestion of caffeine the total carbohydrate oxidation was not appreciably different than in those in which creatinuria did not occur, or in the control experiments in which water alone was taken. After the ingestion of 3 mgm. and 6 mgm. caffeine per kilo there was a marked rise in the respiratory quotient during the first and second periods after ingestion, but this rise was due to hyperventilation and not to an increase in carbohydrate metabolism (4). It was later followed by a fall of the quotient below the base line because of a compensatory retention of carbon dioxide. Notwithstanding the hyperventilation, calculations of the amount of carbohydrate oxidized on the basis of the respiratory exchange for the entire post-ingestion period of $1\frac{3}{4}$ hours were doubtless correct inasmuch as the compensation by retention of carbon dioxide was completed at the end of this period.

*Creatinuria and nitrogen excretion.* The procedure followed in these experiments was the same as in numerous experiments on metabolism conducted in our laboratories. Under these conditions we have found that the nitrogen excretion is almost invariably less in the post-experimental than in the post-absorptive period. Inspection of the average excretion in the different groups of the caffeine experiments indicated a trend toward smaller differences between the post-absorptive and post-experimental excretion of nitrogen as the dosage of caffeine was increased. A statistical analysis of the data showed that in those experiments in which creatine was found in the urine the decline in nitrogen excretion during the post-experimental period was significantly less than when there was no creatine present. In the experiments with no creatine in the urine, the average hourly nitrogen excretion was 108 mgm. less in the post-experimental than in the post-absorptive period whereas in the experiments with creatinuria it was 15 mgm. less. The difference between these two values (93 mgm.) was statistically significant as it was four times the sigma of the difference.

Discussion. Since there was no relationship in these experiments between the presence or the amount of creatine in the urine and the oxygen utilization or carbon dioxide elimination, it appears that the underlying mechanism responsible for creatine excretion following the ingestion of caffeine is not related to an increase in total metabolism. In view of these findings, may not the creatinuria observed in hyperthyroidism (cf. 8, p. 247) be merely incidental and not due to the heightened metabolism?

Brentano (3) found that the injection of 2 mgm. caffeine per kilo body weight

into rabbits was followed by creatinuria which paralleled a diminution in the glycogen content of the skeletal muscles. From these and other experiments he concluded that those conditions which lead to a reduction in muscle glycogen result in the excretion of creatine. Our experiments offer no direct evidence with regard to the glycogen content of the muscle before and after the administration of caffeine, but if a diminution in muscle glycogen did occur, it was not reflected either in a rise in blood lactic acid (5) or in an increased oxidation of carbohydrate.

Creatinuria induced by the ingestion of caffeine could conceivably result from an increase in the rate of blood flow with the consequent washing out of creatine from the tissues. Although the rate of blood flow in these experiments is not known, in view of the results obtained by Beard and his associates on rats (1, 9) it may be assumed that this factor was not responsible for the creatinuria. These observers found that after parenteral injection of caffeine the increase in the amount of creatine in the urine was accompanied by an increase and not by a decrease in the creatine content of the muscle. Creatinuria could perhaps be the consequence of diuresis but this did not occur in our experiments. In all the experiments the hourly excretion of urine was less in the post-experimental than in the post-absorptive period.

The larger amount of nitrogen in the post-experimental relative to the post-absorptive urine that was found when creatinuria occurred, could have resulted from an increase in the total volume of urine excreted or from stimulation of protein metabolism. Analysis of the data showed that there was no significant difference in the total volume of urine in the experiments with and without creatinuria. In the former the average hourly excretion was 14 cc. less in the post-experimental than in the post-absorptive period, while in the latter it was 56 cc. less. The difference between these two values was only 1.8 times the sigma of the difference. The difference between the nitrogen excretion in the experiments with and without creatinuria was statistically significant. Likewise, the difference between the excretion in the control experiments with water and with caffeine when the dosage exceeded 1 mgm. per kilo was significant. It appears therefore that the smaller difference between the post-absorptive and post-experimental excretion of nitrogen associated with creatinuria must be attributed to a stimulation of protein metabolism by caffeine.

The increase in protein metabolism may have been merely coincidental with the creatinuria or may have been a causative factor. While no definite conclusion can be drawn from the data at hand, it appears probable that the effects produced by caffeine on protein metabolism and creatine formation (or elimination) may represent two unrelated actions of the drug. This conclusion is suggested by a comparison of the results of these experiments with those obtained after the ingestion of 50 grams glucose or the same amount of fructose (10, 11). Creatinuria was induced almost invariably by the ingestion of fructose but rarely by the same amount of glucose. There was, however, no increase in protein metabolism (nitrogen excretion) in those experiments in which there was creatinuria, as compared with those in which no creatine was excreted.

As stated previously, there was no correlation in these experiments with

caffeine between the amount or presence of creatine in the urine and the total amount of carbon dioxide eliminated. However, after administration of the larger doses of caffeine which usually produced creatinuria, there was a blowing off of preformed carbon dioxide which was compensated for by retention before the conclusion of the experiment (4). In the experiments with fructose there was likewise a loss of non-metabolic carbon dioxide as a result of the formation of lactic acid (5). In both instances it may be reasonably assumed that there was a temporary alkalosis of the tissues. This common factor in the experiments with caffeine and with fructose would suggest that alkalosis may operate as a fundamental mechanism in the formation or liberation of creatine. It must be admitted, however, that different mechanisms might account for creatine formation under various experimental and pathological conditions.

## CONCLUSIONS

The administration of sufficiently large doses of caffeine was followed by the excretion of creatine in the urine.

The minimal dose of the drug that induced creatinuria was 1 mgm. per kilo, but with this amount creatine was excreted in only 3 out of 14 experiments. With doses of 1.8 to 6.0 mgm. caffeine per kilo, creatine was found in the urine in 30 out of 35 experiments.

The amount of creatine excreted showed no relationship to the amount of caffeine ingested.

There was no correlation between the amount of creatine in the urine and total oxygen consumption and carbon dioxide elimination; nor was there any relationship between the occurrence of creatinuria and the gaseous exchange.

The presence of creatine in the urine was not related to an increase in carbohydrate oxidation.

An increase in protein metabolism was associated with creatinuria but it appears that it was not a causative factor.

While it is recognized that different mechanisms might account for creatine formation under various experimental and pathological conditions, these experiments in conjunction with others on fructose suggest that alkalosis might play a significant rôle in some instance.

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# THE MOTOR INNERVATION OF THE COLON

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The response of the colon to stimulation of its various motor nerves has been the subject of many investigations. An adequate summary of the existing information is to be found in the review by Garry (1). Most of these studies have been concerned primarily with the type of activity (i.e., contraction or relaxation) of the colon which occurs in response to nerve stimulation. However, there are a number of equally important aspects of the motor innervation of the colon about which there is little or no information.

Certain of these issues are as follows: 1. The chemical mediators of the nerve impulse from the various autonomic nerves to the musculature of the colon have not adequately been determined. Such information is necessary for a proper functional classification of these nerves (i.e., as cholinergic, adrenergic, or mixed), and for a proper interpretation of the action of autonomic drugs on the colon. 2. Very little evidence concerning the region of the colon which responds to stimulation of a given nerve, or the direction of the response (circular, longitudinal, or both) is to be found. 3. The pelvic nerves reach the colon deep in the pelvis, but the pathway by which pelvic nerve impulses influence upper levels of the colon is unknown. 4. It is stated (2) that pelvic nerve stimulation causes rapid contraction of the urinary bladder followed by slow relaxation, and it is claimed that atropine does not affect the height of this response but appreciably shortens the period of relaxation. It is unknown whether a similar situation exists in the colon.

The purpose of the present investigation was to supply evidence on these aspects of the motor innervation of the colon.

METHODS. Dogs, pigs and monkeys have been used in the present studies. All animals were anesthetized. Sodium pentobarbital has been the principal anesthetic agent, but ether has been employed in certain instances. The pelvic nerves, the hypogastric nerves (both central and peripheral), the vagus nerves, and the coeliac root of the inferior mesenteric ganglia have all been separately subjected to simulation. The stimulus was delivered to the nerve via fixed, shielded electrodes employing a Harvard inductorium and two 4 volt dry cells as the source of current. The inductorium was set at 4.5 to 8.0 cm., and the stimulus was continued for periods of 2 sec. to 2 min. Impulses were delivered at the rate of 60/sec.

The response of the colon to stimulation of various nerves has been recorded by two mechanical systems. The first, the "balloon-enterograph" system was employed to differentiate activity of a circular from that of a longitudinal nature. This system consisted of a balloon inside the colon, and the movable

arm of a Wiggers myocardiograph sewed to the outside of the colon. The myocardiograph was fixed to a ringstand so that changes in this system are a measurement of longitudinal movement of the colon into and out of the pelvis. A water manometer was employed with the balloon system, and a Becker tambour was used with the enterograph.

The second system was a multiple balloon system, and was used to determine which portions of the colon respond to stimulation of a given nerve, and whether or not progressive involvement of the colon existed. One balloon was placed in the proximal colon, one in the middle and one in the distal colon. Each balloon was attached to a water manometer.

In addition to a sample tracing illustrating any given point, a technique has been devised for measuring the extent of a given response of the colon in quantitative terms. This technique involves the measurement, by means of a planimeter, of the area (sq. mm.) enclosed beneath a given response on the kymograph tracing. This area (sq. mm.) divided by the length of the base line over which the response took place (mm.) gives the average height of the response (mm.). The actual measurement of the average height and the duration of a response provides the essential requirements for a comparison of separate responses in a single animal or for obtaining the average response of a series of animals.

In order to determine for a given response the nature of the chemical mediator of the nerve impulse, various autonomic drugs have been employed. These drugs were the mimetic agents acetyl choline bromide (0.01 mgm./K) and epinephrine hydrochloride (0.008–0.011 mgm./K); the blocking agents ergotamine tartrate (0.02–0.03 mgm /K) and atropine sulfate (0.44 mgm./K); and the potentiating agents physostigmine salicylate (0.5 mgm./K) and cocaine hydrochloride (5.0 mgm./K). The acetyl choline and the epinephrine were injected into the aorta just above the origin of the inferior mesenteric artery, and their effect on the colon was noted. The atropine and ergotamine were injected intravenously, and the physostigmine and cocaine were injected subcutaneously. Nerve stimulation of constant strength and duration was conducted before and after the administration of the various blocking and potentiating drugs.

In the studies on the pathway by which pelvic nerve impulses influence upper levels of the colon the following procedures were employed. Two balloons were placed in the colon, one in the distal and one in the descending colon. In one series of experiments the colon was transected between the balloons, and then reanastomosed. In the other series the wall of the colon between the balloons was infiltrated with 2 per cent cocaine.

Results. 1. *Pelvic nerve.* Stimulation of the pelvic nerve caused a contraction of the colon of the dog (37 out of 41 animals); monkey (3 out of 4 animals); and the pig (4 out of 5 animals). Visual inspection of the colon during the course of pelvic nerve stimulation revealed a rather marked longitudinal shortening of the colon. This longitudinal contraction appeared to originate at the most distal end of the colon and gradually involved higher and higher seg-

ments. The net result was a progressive drawing down of the colon into the pelvis. Coincident with the shortening of the colon it appeared to decrease in diameter and became very firm to the touch. A balloon lying free in the distal colon was expelled by this action.

By means of the "balloon-enterograph" system it was observed that this contraction of the colon occurred in both a longitudinal and a circular direction (table 1). However, the relative degree of involvement of the two muscular coats was not constant. Inspection of the mechanograms of this response (fig. 1, A) revealed that it consisted of a rapid contraction followed by a somewhat slower relaxation phase.

Employing the multiple balloon system it was observed (table 2) that in 10 out of 11 dogs the response was confined to the descending and distal colon. In the one remaining animal a rather large response of the proximal colon was noted. The average figures presented in table 2 are, therefore, somewhat misleading. A typical tracing of this response is shown in figure 1, B-1. It can be seen that the response was greatest in the distal colon.

TABLE 1

*Type of response of the colon to stimulation of the various nerves*

| NERVE STIMULATED | NO. DOGS | STIMULATION | | | RESPONSE | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Circular | | Longitudinal | |
| | | No. | Ave. strength | Ave. duration | Ave. height | Ave. dura. | Ave. height | Ave. dura. |
| | | | *cm.* | *sec.* | *mm.* | *min.* | *mm.* | *min.* |
| Pelvic | 28 | 61 | 7.0 | 7.5 | 6.3 | 2.40 | 6.1 | 2.05 |
| Hypogastric | 15 | 34 | 4.7 | 26.6 | 5.6 | 4.55 | −0.3* | 0.09 |
| Coeliac root of inf. mes. ganglia | 10 | 29 | 5.0 | 30.0 | 7.7 | 3.84 | −0.5* | 0.92 |

* Indicates movement of the colon out of the pelvis in a few animals.

In a series of 5 dogs it was observed that the response of the descending, but not the distal colon was abolished by transection between these two portions of the colon. The response of the descending colon was not reconstituted by anastomosis of the severed ends of the colon. In order to determine whether the abolition of the response of the descending colon was due to the removal of a mechanical or a nervous influence, 2 per cent cocaine was infiltrated into the wall of the colon between the balloons. In a series of 4 dogs it was observed that such a procedure abolished the response of the descending but not the distal colon. If adequate time was allowed for the cocaine to be absorbed and removed then the response of the descending colon again occurred (fig. 1, C).

In a series of 10 dogs it was shown that physostigmine potentiated the response of the colon to pelvic nerve stimulation (table 3-2). If a constant stimulus was applied to the pelvic nerve before and 10–15 min. after the subcutaneous injection of physostigmine it was possible to show potentiation of the response of the colon to pelvic nerve stimulation without any direct stimulation of the colon by the drug itself (fig. 1, D). If more than 15 min. elapsed follow-

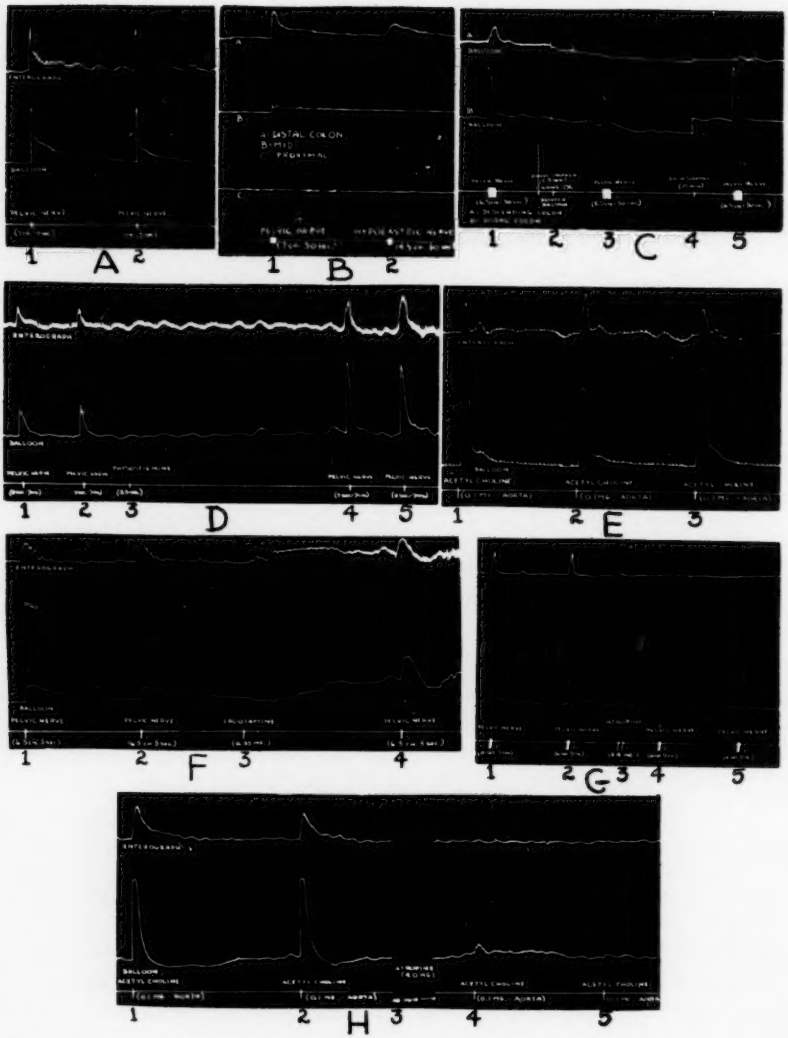


Fig. 1. A (1, 2) pelvic n. stim.; upper, long.; lower, circ.

B (1) pelvic n. stim. (2) hypogastric n. stim.; in descending order balloons in dist. colon, mid. colon, prox. colon.

C (1, 3, 5) pelvic n. stim.; (2) cocaine in wall of colon; (4) drum stopped 15 min. upper line, balloon in descend. colon; lower line, balloon in dist. colon.

D (1, 2, 4, 5) pelvic n. stim.; (3) subcut. physostigmine; upper, long.; lower, circ.

E (1, 2, 3) intra-art. acetyl choline; upper, long.; lower, circu.

F (1, 2, 4) pelvic n. stim.; (3) intraven. ergotamine; upper, long.; lower, circ.

G (1, 2, 4, 5) pelvic n. stim.; (3) intraven. atropine; upper, long.; lower, circ.

ing the injection there was an increase in circular and longitudinal activity of the colon due to the physostigmine.

The intra-arterial injection of acetyl choline was shown in 10 dogs to cause both circular and longitudinal contraction of the colon (table 3-3). The response was similar to that obtained as a result of pelvic nerve stimulation. If the acetyl choline was injected into the aorta just above the origin of the inferior mesenteric artery uniform results were obtained (fig. 1, E). It was apparent that the circular contraction of the colon was somewhat greater following acetyl choline injection than following pelvic nerve stimulation, and the longitudinal contraction was somewhat less.

In 3 dogs the effect of ergotamine on the response of the colon to pelvic nerve stimulation was determined. It was found (fig. 1, F) that ergotamine is without appreciable effect on this response.

It was apparent in 18 dogs that atropine inhibited the response of the colon to pelvic nerve stimulation (table 3-1). If a constant stimulus was applied to the pelvic nerve before and after the intravenous injection of atropine, marked

TABLE 2

*Location of the response of the colon to stimulation of various nerves*

| NERVE STIMULATED | NO. DOGS | STIMULATION | | | RESPONSE AREA OF THE CONTRACTION CURVE (SQ. CM.) | | |
|---|---|---|---|---|---|---|---|
| | | No. | Strength (ave.) | Duration (ave.) | Proximal colon | Descending colon | Distal colon |
| | | | *cm.* | *sec.* | | | |
| Pelvic | 11 | 24 | 6.5 | 15 | 0.32* | 0.71 | 2.67 |
| Hypogastric | 7 | 20 | 4.4 | 30 | 0.00 | 0.00 | 0.97 |
| Coeliac root of the inf. mes. ganglia | 10 | 28 | 5.0 | 30 | 0.00 | 3.70 | 0.00 |

* Indicates large involvement of proximal colon in only one animal.

inhibition of the response occurred (fig. 1, G). In 7 out of 18 dogs the present dose of atropine completely abolished the response of the colon to pelvic nerve stimulation. In 1 dog the longitudinal response was abolished but some circular action persisted, and in 1 dog the circular response was abolished but some longitudinal action persisted. In the remaining 9 dogs there was a residue of both types of action following atropine. The total per cent inhibition of longitudinal and circular activity was the same.

In those animals in which a residual response remained after 0.44 mgm./K. of atropine, it was observed that multiples of this dose were ineffective in bringing about abolition of the residue. It was observed in 10 dogs that atropine was very effective in inhibiting the response of the colon to the intra-arterial injection of acetyl choline (table 3-3b). If the same dose of acetyl choline was injected before and after atropine, it was apparent that the response was inhibited by atropine (fig. 1, H). In 3 dogs both circular and longitudinal responses were abolished by atropine; in 2 dogs only the circular; in 3 dogs only

the longitudinal; and in 1 dog neither longitudinal nor circular responses were completely abolished by this dose. It was apparent (table 3) that there was a 97.5 per cent inhibition of the response of the colon to injected acetyl choline by 0.44 mgm./K. of atropine, but only an 88.6 per cent inhibition of the response to pelvic nerve stimulation by the same dose of atropine.

TABLE 3

*The effect of various autonomic drugs on the response of the colon*

| PROCEDURE | STIMULATION | | | DOSE | ROUTE | SERIES | NO. DOGS | RESPONSE | | | | PER CENT CHANGE* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Circular | | Longitudinal | | | |
| | No. | Ave. strength | Ave. duration | | | | | Ave. height | Ave. duration | Ave. height | Ave. duration | Circular | Longitudinal |
| | | *cm.* | *sec.* | *mgm. per kgm.* | | | | *mm.* | *min.* | *mm.* | *min.* | | |
| 1. Pelvic nerve stim. | 78 | 7.0 | 10.0 | | | A. Control | 18 | 6.2 | 2.82 | 5.6 | 2.26 | −88.9 | −88.2 |
| | | | | | | B. Atropine† | | 1.7 | 1.14 | 1.9 | 0.79 | | |
| 2. Pelvic nerve stim. | 44 | 7.0 | 5.0 | | | A. Control | 10 | 6.4 | 1.67 | 7.1 | 1.68 | +135.7 | +127.3 |
| | | | | | | B. Physostig.† | | 10.9 | 2.31 | 12.6 | 2.15 | | |
| 3. Acetyl choline inj. | 42 | | | 0.01 | Intra-arterial | A. Control | 10 | 10.2 | 3.30 | 3.2 | 2.40 | −97.3 | −97.6 |
| | | | | | | B. Atropine† | | 1.0 | 0.90 | 0.6 | 0.30 | | |
| 4. Hypogastric nerve stim. | 32 | 4.7 | 30.0 | | | A. Control | 7 | 5.3 | 4.74 | −0.7‡ | 0.21 | −118.3 | +356.2 |
| | | | | | | B. Ergotam.† | | −2.5 | 1.85 | −1.6‡ | 0.42 | | |
| 5. Hypogastric nerve stim. | 16 | 4.5 | 25.0 | | | A. Control | 4 | 4.0 | 4.66 | 0.0 | 0.00 | +28.3 | |
| | | | | | | B. Cocaine† | | 4.3 | 5.57 | 0.0 | 0.00 | | |
| 6. Epinephrine injection | 42 | | | 0.08-0.11 | Intra-arterial | A. Control | 10 | 11.1 | 6.39 | −0.8‡ | 0.89 | −123.4 | +1643.2 |
| | | | | | | B. Ergotm.† | | −3.7 | 4.49 | −3.9‡ | 3.19 | | |
| 7. Hypogastric nerve stim. | 20 | 5.0 | 25.0 | | | A. Control | 4 | 7.6 | 6.11 | 0.0 | 0.00 | +137.9 | |
| | | | | | | B. Atropine† | | 13.6 | 8.13 | 0.0 | 0.00 | | |
| 8. Stim. of the coeliac root of the inf. mes. ganglia | 58 | 5.4 | 30.0 | | | A. Control | 6 | 7.0 | 3.60 | −0.4‡ | 0.93 | −115.8 | +132.8 |
| | | | | | | B. Ergotam.† | | −3.1 | 1.29 | −1.2‡ | 0.72 | | |

* Change in the average area of the response curve. Ave. ht. × (Ave. Duration/0.8) (1 min. = 0.8 cm. on kymograph drum).

† After.

‡ Indicates movement of the colon out of the pelvis.

It is evident from table 3 that atropine produced inhibition of both the average height and the duration of the response of the colon to pelvic nerve stimulation.

2. *Vagus nerve.* Stimulation of the vagus nerve, both in the neck and on the esophagus just above the diaphragm, was uniformly ineffective in producing a response of the colon of the dog (10 animals). A slight but inconstant contraction of a portion of the cecum was noted in 1 out of 5 pigs, and in 1 out of 4 monkeys. In the remainder of these latter animals no response of the colon to

vagus nerve stimulation was noted. It was not possible to record the contraction in the pig and monkey colon by balloon techniques. The response appeared to be confined to a portion of the taenia, and slight approximation of silk ligatures tied to the taenia was observed.

3. *Hypogastric nerve.* Stimulation of the peripheral hypogastric nerve produced a response of the colon in only 15 out of 32 dogs. However, when a response was observed it was purely motor. On visual inspection, the response consisted of one or more isolated and stationary rings of constriction. There was no apparent movement of the colon in a longitudinal direction.

By means of the "balloon-enterograph" system it was observed that the contraction of the colon following hypogastric nerve stimulation was primarily circular in nature (table 1). In 2 out of 15 animals there was slight depression of the longitudinal recorder, indicating a movement of the colon out of the pelvis. In general, however, the response was a simple circular contraction, which developed rather slowly and regressed rather slowly (fig. 2, A).

Employing the multiple balloon system it was observed in 7 dogs (table 2) that the response of the colon to hypogastric nerve stimulation was confined to the distal colon. Unlike the response to pelvic nerve stimulation there was no response of the descending colon (fig. 1, B-2).

It was shown in 7 dogs that ergotamine tartrate was effective in inhibiting the circular contraction of the colon in response to hypogastric nerve stimulation (table 3-4). In 2 dogs the response was abolished. In the remaining 5 dogs there was reversal of the response, and after ergotamine a circular relaxation occurred (fig. 2, C). In 1 animal in this series there was a depression of the longitudinal recorder before ergotamine. After ergotamine in this animal the depression was augmented, indicating an increased movement of the colon out of the pelvis. In another animal in this series (fig. 2, C), whereas there was no longitudinal activity before ergotamine, after ergotamine there was movement of the colon out of the pelvis.

The effect on the colon of the intra-arterial injection of epinephrine was determined in 10 dogs. It was shown (table 3-6) that epinephrine caused a circular contraction in all cases. In 2 out of the 10 dogs there was a depression of the longitudinal recorder indicating a movement of the colon out of the pelvis. In the remaining 8 dogs there was a circular contraction of the colon with no appreciable longitudinal response (fig. 2, E). The effect of ergotamine on this response was determined on the above animals (table 3-6). In all 10 animals the circular contraction was reversed and appeared as a relaxation. In the 2 animals in which previous to ergotamine there had been a longitudinal movement of the colon out of the pelvis, this movement was increased following ergotamine. In 6 of the remaining animals in which no longitudinal action was noted before ergotamine, after ergotamine there was a slight movement of the colon out of the pelvis (fig. 2, H).

The effect of cocaine on the response of the colon to hypogastric nerve stimulation was determined in 4 dogs. It was observed that there was slight potentiation of the circular contraction (table 3-5).

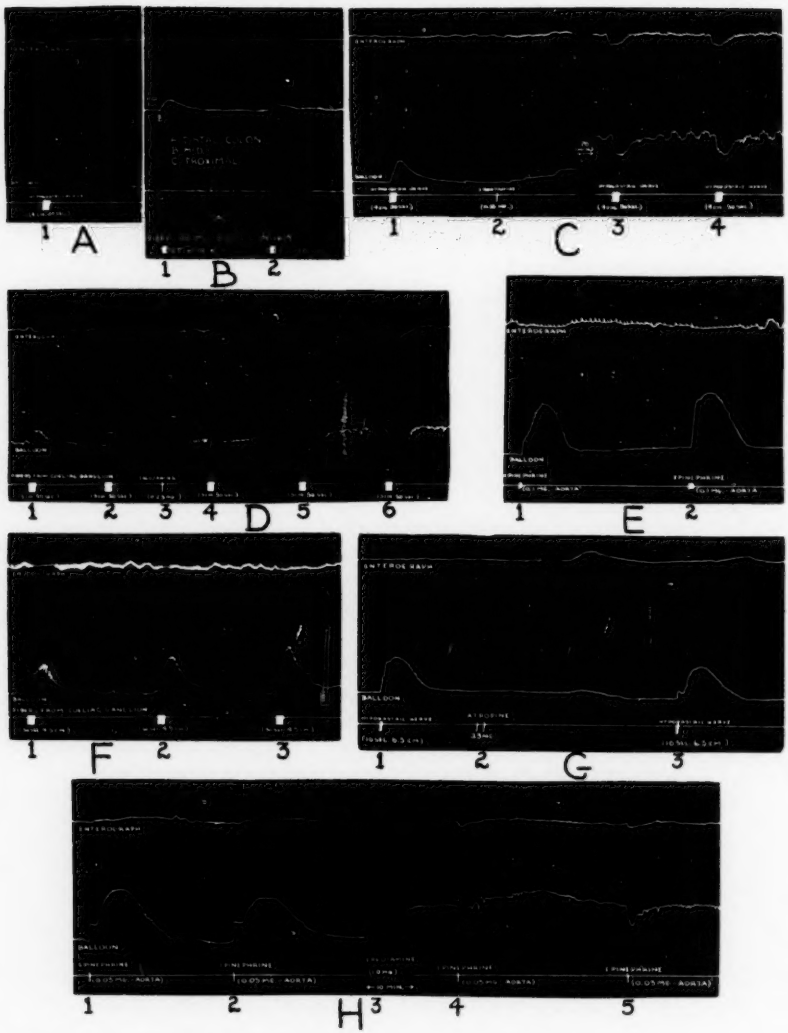


Fig. 2. A (1) hypogastric n. stim.; upper, long.; lower, circ.

B (1, 2) stim. of fibers to coeliac gang. In descend. order, balloons in dist. colon, mid. colon, prox. colon.

C (1, 3, 4) hypogastric n. stim. (2) intraven. ergotamine; upper, long.; lower, circ.

D (1, 2, 4, 5, 6) stim. of fibers to coeliac gang. (3) intraven. ergotamine; upper, long.; lower, circ.

E (1, 2) intra-art. epinephrine; upper, long.; lower, circ.

F (1, 2, 3) stim. of fibers to coeliac gang.; upper, long.; lower, circ.

G (1, 3) hypogastric n. stim. (2) intraven. atropine; upper, long.; lower, circ.

H (1, 2, 4, 5) intra-art. epinephrine (3) intraven. ergotamine, followed by a 10-minute interval; upper, long.; lower, circ.

The effect of atropine on the response of the colon to hypogastric nerve stimulation was determined in 4 dogs. It was apparent that the present dose of atropine did not inhibit the response (table 3-7). If a constant stimulus was applied to the hypogastric nerve before and after atropine, it was observed that there was no appreciable change in the response (fig. 2, G).

Stimulation of the central end of the cut hypogastric nerve was ineffective in producing a response of the colon in a series of 10 dogs.

4. *Coeliac root of the inferior mesenteric ganglia.* Stimulation of those fibers which connect the upper collateral ganglia with the inferior mesenteric ganglia usually produced a response of the colon (15 out of 20 dogs). On visual inspection of the colon this response, like that to hypogastric nerve stimulation, consisted of one or more isolated and stationary rings of constriction.

By means of the multiple balloon system it was observed in 10 dogs that this contraction was confined to the descending colon (table 2). There was no involvement of either the proximal or the distal colon (fig. 2, B).

Employing the "balloon-enterograph" system this response was recorded in 10 dogs (table 1). It was apparent that a circular contraction occurred in all animals. In 1 out of 10 dogs there was depression of the longitudinal recorder indicating movement of the colon out of the pelvis. In general, however, the response consisted of a simple circular contraction of the colon with no change in the longitudinal direction (fig. 2, F).

The effect of ergotamine on the response of the colon to stimulation of the coeliac root of the inferior mesenteric ganglia was determined in a series of 6 dogs (table 3-8). In 3 out of the 6 animals the circular contraction became a relaxation following ergotamine; in 1 animal the contraction was abolished; and in 2 animals it was only inhibited. In the 1 animal which showed depression of the longitudinal recorder before ergotamine, after ergotamine the movement out of the pelvis was increased (fig. 2, D). In another animal in which there was no longitudinal activity before ergotamine, after ergotamine there was movement of the colon out of the pelvis.

It has been shown in experiments on 3 dogs that atropine was without appreciable effect on the present response.

Discussion. The pelvic nerves to the musculature of the colon appear to be cholinergic, as evidenced by the observations that, in general, the response of the colon to pelvic nerve stimulation is inhibited by atropine; potentiated by physostigmine; not inhibited by ergotamine; and mimicked by the injection of acetyl choline.

It is apparent from the present evidence that the response of the colon to pelvic nerve stimulation differs in part from the response to injected acetyl choline. This difference lies in the fact that the injected acetyl choline produces a greater circular response of the colon but a lesser longitudinal response than that produced by pelvic nerve stimulation. It is possible that this discrepancy could be explained by an unequal distribution of nerve fibers to the two muscle layers. If more nerve fibers pass to longitudinal than to circular muscle fibers, then the ratio of longitudinal to circular activity would be high following pelvic nerve stimulation due to a higher concentration of acetyl choline in the longitu-

dinal layer. On the other hand, the injected acetyl choline would have equal access to both muscle groups. This notion assumes that the increased circular response to injected acetyl choline is due to a higher effective concentration of acetyl choline than that produced by pelvic nerve stimulation.

In the present studies it has also been observed that atropine is more effective in inhibiting the response to injected acetyl choline than it is in inhibiting the response to pelvic nerve stimulation. The evidence of Hill (3) indicates that autonomic nerves may enter into smooth muscle cells. If this is true then the present phenomenon may be explained by the theory of Dale and Gaddum (4). This theory states that atropine acts only at cell surfaces and will prevent the action of acetyl choline which must enter the cell, but is without effect upon the action of acetyl choline which is liberated within the cell. If it is assumed that not all the muscle fibers of the colon are directly innervated and that many of them respond to acetyl choline which diffuses from the innervated cells, then it is apparent that atropine would prevent the response of the noninnervated cells, but would not completely abolish the response to pelvic nerve stimulation.

It has been suggested that pelvic nerve fibers extend upward in the hypogastric nerves (5, 6). It might appear that this was the pathway by which pelvic nerve impulses reach the upper portion of the colon. However, in view of the present evidence 1, that stimulation of the central end of the cut hypogastric nerves is without effect on the colon, and 2, that the response of the descending but not the distal colon is abolished by transection or cocainization between these two portions of the colon, it would appear that the most important route of such impulses lies in the wall of the colon.

Henderson and Roepke (2) claim that in the urinary bladder pelvic nerve stimulation causes a dual contractile and tonus response. They state that only the tonus or slow relaxation phase of this response is affected by atropine. The present observation that both the average height and the duration of the response of the colon to pelvic nerve stimulation are inhibited by atropine would indicate that a similar mechanism does not exist in the colon.

The notion that the sympathetic nerves may cause contraction of the colon is not a new one. It has been claimed to occur at times by others (7-10). However, the possibility that epinephrine could cause contraction of the colon was not investigated prior to the studies of Templeton and Lawson (11). The present observations on the motor action of epinephrine on the colon, as part of the proof of the adrenergic nature of the motor effect of the sympathetic nerves on the colon are in general agreement with those of Templeton and Lawson. However, in the present study the secondary increase in longitudinal activity of the colon noted by these investigators following the injection of epinephrine has not been observed.

The explanation for the movement of the colon out of the pelvis occasionally observed following sympathetic nerve stimulation is not apparent. It is unknown whether this movement is due to a longitudinal relaxation below the recorder or a longitudinal contraction above the recorder. However, the abrupt nature of the response suggests that longitudinal contraction occurs above the recorder.

This movement was not abolished by ergotamine. In fact, it was potentiated by this drug. However, this finding does not imply a cholinergic component in the hypogastric nerves, in view of the observation that similar responses were noted following the injection of epinephrine. This longitudinal movement is not explained on the basis of an artifact due to rather strong circular constriction of the colon, as it was observed that reversal of the circular response by ergotamine did not lessen this longitudinal action.

## CONCLUSIONS

1. The pelvic nerves to the musculature of the colon are cholinergic. It is suggested that unequal distribution of pelvic nerve fibers to the muscle layers, and the intracellular ending of certain pelvic nerve fibers explain the discrepancies between the response of the colon to pelvic nerve stimulation and that to the injection of acetyl choline. Electrical stimulation of the pelvic nerves causes longitudinal and circular contraction of the descending and distal colon. These nerves influence upper levels of the colon via nerve pathways located in the wall of the colon. A dual contractile and tonus mechanism in response to pelvic nerve stimulation does not exist in the colon as it has been claimed to exist in the urinary bladder.

2. Electrical stimulation of the vagus nerve is ineffective in producing a response of the colon of the dog. However, in certain instances it may produce a weak and inconstant contraction of a portion of the cecum in the pig and monkey.

3. The hypogastric nerves to the musculature of the colon are adrenergic. Electrical stimulation causes a circular contraction which is confined to the distal colon. This effect, however, is not uniformly obtained.

4. The fibers of the coeliac root of the inferior mesenteric ganglia which act on the colon are adrenergic. When they are stimulated electrically a circular contraction which is confined to the descending colon results. This effect, however, is not uniformly obtained.

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# RENAL EXCRETION OF POTASSIUM SALTS[1,2]

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The renal excretion of both cations and anions following the injection of various salts of a single typical anion, sulfate, has been the subject of a previous report (13). Certain characteristics of the renal excretion of electrolytes were defined on the basis of these experiments. The present study deals in a similar way with the excretion of cations and anions following the injection of different salts of a single cation, potassium. Any modification of the excretion of potassium could be determined by comparing one experiment with another. Any conditioning of the excretion of anions by potassium could also be studied, at least insofar as data are available through their excretion with cations other than potassium.

METHODS AND MATERIALS. Experiments were conducted with dogs, using multiple successive periods of urine collection and several blood samples before and after injection of potassium salts. The exact procedure has been described elsewhere (13). Creatinine clearance determined in all periods after injection of the salt was assumed to be identical with glomerular filtration rate in the dog (14, 15).

Potassium and sodium were determined by the method of Hald (9), sulfate by the method of Cope (3), phosphate by the method of Fiske and Subbarow (5), chloride by the method of Van Slyke (11). Bromide was determined first by the method of Hastings and Van Dyke (10) and later by the method of Brodie and Friedman (2). pH of urine was determined, using a Hastings compensating colorimeter and suitable indicators (11). With urines of a high pH and a high bicarbonate content the urine was collected directly from the catheter into a glass Ostwald pipette with a stopcock. Bicarbonate of urine was calculated from the total carbon dioxide content of the urine, determined by the method of Van Slyke (11), and the pH of the urine.

RESULTS. In table 1 are presented the data from six experiments in which potassium chloride alone was injected, while in table 2 are the data from five experiments in which potassium bromide alone was injected. In table 3 are recorded the protocols of four experiments in which potassium bromide and neutral potassium phosphate were injected together. Data from two experiments following the injection of potassium sulfate have been presented in detail in a previous paper (13), and so are not reproduced here. These results will be analyzed in various ways.

[1] Aided by grants from the Ella Sachs Plotz Fund, the Emerson Fund, and the Fluid Research Fund of the Yale University School of Medicine.

[2] A preliminary report of part of this work was presented before the American Physiological Society in 1940: This Journal **129:** P498, 1940.

TABLE 1

*Intravenous injection of isotonic potassium chloride solution*

| EXPERIMENT NUMBER | DOG WEIGHT | SALT GIVEN | PERIOD | DURATION | URINE FLOW | CONCENTRATION (M. EQ. PER LITER) | | | | | | CLEARANCES (CC. PER MINUTE) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Urine | | | | Serum | | | |
| | | | | | | $K^+$ | $Na^+$ | $Cl^-$ | $HCO_3^-$ | $K^+$ | $Cl^-$ | $K^+$ | Creatinine |
| | *kgm.* | *mM.* | | *minutes* | *cc. per minute* | | | | | | | | |
| 1 | 20.5 | 60.8 | | | | | | | | 3.1 | 106.3 | | |
| | | | 1 | 59 | 0.61 | 26 | | 52 | | | | 5 | |
| | | | 2* | 167 | 0.66 | 223 | | 230 | | | | | |
| | | | | | | | | | | 6.2 | 106.8 | | |
| | | | 3 | 75 | 0.36 | 400 | | 219 | | | | 26 | 92 |
| | | | | | | | | | | 4.4 | 107.3 | | |
| | | | 4 | 60 | 0.22 | 386 | | 136 | | | | 18 | 84 |
| | | | | | | | | | | 4.8 | 106.4 | | |
| 2 | 23.2 | 72.5 | | | | | | | | 3.9 | 105.7 | | |
| | | | 1 | 18 | 0.17 | 41 | | 12 | | | | 2 | |
| | | | 2* | 136 | 0.88 | 244 | | 217 | | | | | |
| | | | | | | | | | | 6.1 | 108.9 | | |
| | | | 3 | 84 | 0.36 | 414 | | 203 | | | | 25 | 102 |
| | | | | | | | | | | 5.6 | 109.0 | | |
| | | | 4 | 46 | 0.22 | 445 | | 150 | | | | 18 | 94 |
| | | | | | | | | | | 5.5 | 107.6 | | |
| 3† | 22.2 | 77.0 | | | | | | | | 7.2 | | | |
| | | | 1 | 33 | 0.30 | 86 | 35 | 96.6 | 164 | | | 3 | |
| | | | 2* | 76 | 1.26 | 155 | 45 | 148.0 | 35 | | | | |
| | | | | | | | | | | 7.8 | | | |
| | | | 3 | 75 | 0.48 | 218 | 10 | 73.8 | 101 | | | 13 | 36 |
| | | | | | | | | | | 8.1 | | | |
| | | | 4 | 120 | 0.33 | 189 | 19 | 34.9 | 54 | | | 8 | 30 |
| | | | | | | | | | | 6.7 | | | |
| 4 | 20.7 | 68.8 | 1* | 78 | 0.49 | 243 | | 97 | 187 | | | | |
| | | | 2 | 72 | 0.38 | 340 | | 106 | 223 | | | | |
| | | | 3 | 137 | 0.13 | 186 | | 21 | 290 | | | | |
| | | | 4 | 1112 | 0.61 | | | 68 | | | | | |
| 5 | 24.2 | 53.6 | 1* | | | 194 | | 177 | 77 | | | | |
| | | | 2 | 50 | 0.62 | 311 | | 180 | 135 | | | | |
| | | | 3 | 153 | 1.64 | 58 | | 28 | 21 | | | | |
| | | | 4 | 1100 | 0.16 | 79 | | 111 | | | | | |
| 6 | 15.0 | 52.0 | 1* | 75 | 0.37 | 243 | | | 164 | 6.6‡ | | | |
| | | | | | | | | | | 6.9‡ | | | |
| | | | 2 | 152 | 0.22 | 413 | | | 325 | | | 11 | |
| | | | | | | | | | | 9.5‡ | | | |
| | | | 3 | 165 | 0.19 | 476 | | | 63 | | | | |

* Indicates period during which injection was made.

† $NH_4$ concentrations in four periods were respectively 190, 16, 11 and 10 mM. per liter urine; $PO_4$ concentrations were respectively 7, 3, 0 and 10 mM. per liter urine.

‡ Serum pH 7.29, 7.32 and 7.30 respectively determined by Dr. T. Rosenthal using a glass electrode; serum bicarbonate 23.9, 23.4 and 27.4 mM. per liter respectively.

TABLE 2

*Intravenous injection of isotonic potassium bromide solution*

| EXPERIMENT NUMBER | DOG WEIGHT | SALT GIVEN | PERIOD | DURATION | URINE FLOW | CONCENTRATION (M. EQ. PER LITER) | | | | | | | | | CLEARANCES (CC. PER MINUTE) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Urine | | | | | | | Serum | | | |
| | | | | | | $NH_4^+$ | $K^+$ | $Na^+$ | $Cl^-$ | $Br^-$ | $HPO_4^=$ | $HCO_3^-$ | $K^+$ | $Cl^-$ | $Br^-$ | K | Creatinine |
| | *kgm.* | *mM.* | | *minutes* | *cc. per minute* | | | | | | | | | | | | |
| 7 | 20.9 | 74.8 | | | | | | | | | | | 4.4 | 106.2 | 0.0 | | |
| | | | 1 | 16 | 0.47 | | 57 | | 106 | 0 | | | | | | 6 | |
| | | | 2* | 133 | 1.58 | | 128 | | 232 | 18 | | | | | | | |
| | | | | | | | | | | | | | 7.5 | 92.2 | 14.2 | | |
| | | | 3 | 57 | 0.42 | | 381 | | 231 | 24 | | | | | | 23 | 83 |
| | | | | | | | | | | | | | 6.5 | 89.8 | 14.3 | | |
| | | | 4 | 64 | 0.31 | | 329 | | 223 | 19 | | | | | | 16 | 72 |
| | | | | | | | | | | | | | 6.6 | 89.2 | 13.9 | | |
| 8 | 23.0 | 80.8 | | | | | | | | | | | 3.2 | 104.8 | 0.0 | | |
| | | | 1 | 27 | 0.17 | | 35 | | 24 | 0 | | | | | | | |
| | | | 2* | 51 | 2.06 | | 250 | | 135 | 14 | | | | | | 5 | |
| | | | | | | | | | | | | | 5.4 | | 14.6 | | |
| | | | 3 | 70 | 0.36 | | 433 | | 81 | 8 | | | | | | 27 | 85 |
| | | | | | | | | | | | | | 6.3 | | 14.1 | | |
| | | | 4 | 65 | 0.28 | | 497 | | 61 | 4 | | | | | | 24 | 99 |
| | | | | | | | | | | | | | 5.6 | 90.3 | 14.3 | | |
| 9 | 17.2 | 48.7‡ | | | | | | | | | | | 5.2 | | | | |
| | | | 1 | 44 | 0.18 | | 59 | 5 | 16 | 0 | 0 | 3 | | | | 2 | |
| | | | 2* | 98 | 0.69 | 23 | 101 | 1 | 28 | 3 | 2 | 27 | | | | 4 | 30 |
| | | | | | | | | | | | | | 8.2 | | | | |
| | | | 3 | 80 | 0.31 | 6 | 112 | 0 | 15 | 3 | 0 | 49 | | | | 2 | 20 |
| | | | | | | | | | | | | | 8.9 | | | | |
| | | | 4 | 122 | 0.21 | 2 | 62 | 1 | 16 | 3 | 2 | 23 | | | | | |
| | | | | | | | | | | | | | 8.7 | | | | |
| 10 | 21.8 | 76.5 | | | | | | | | | | | 6.1 | 110.1 | 0.0 | | |
| | | | 1 | 12 | 0.83 | 13 | 44 | 22 | 48 | 0 | | 14 | | | | 6 | |
| | | | 2* | 83 | 0.95 | | | 75 | 130 | 9 | | | | | | | |
| | | | | | | | | | | | | | 11.3 | 101.3 | 11.7 | | |
| | | | 3 | 68 | 0.66 | 4 | 324 | 42 | 159 | 11 | 2 | 83§ | | | | 22 | 65 |
| | | | | | | | | | | | | | 8.0 | 103.4 | 11.6 | | |
| | | | 4 | 106 | 0.37 | 11 | 344 | 21 | 123 | 8 | 46 | 59§ | | | | 15 | 56 |
| | | | | | | | | | | | | | 9.0 | 101.0 | 11.2 | | |
| 11 | 13.0 | 57.1 | | | | | | | | | | | 5.2 | 95.6 | 0.4 | 3 | |
| | | | 1 | 34 | 0.50 | 4 | 37 | 8 | 7 | 0 | 0 | 2† | | | | | |
| | | | 2* | 74 | 1.76 | | 163 | 54 | 50 | 6 | 0 | 118§ | | | | | |
| | | | | | | | | | | | | | 7.5 | 83.0 | 14.8 | | |
| | | | 3 | 52 | 0.52 | 3 | 177 | 4 | 17 | 2 | 0 | 90§ | | | | 13 | 42 |
| | | | | | | | | | | | | | 6.2 | 84.6 | 14.2 | | |
| | | | 4 | 118 | 0.35 | 8 | 122 | 6 | 9 | 1 | 54 | 17 | | | | 8 | 35 |
| | | | | | | | | | | | | | 5.2 | 86.3 | 13.7 | | |

* Indicates period during which injection was made.
† Millimols multiplied by 2.
‡ Injected in 500 cc., therefore a hypotonic solution.
§ May be low due to loss of carbon dioxide.

A. *Relationship between urinary excretion of potassium and the concentration of potassium in serum.* In figure 1 excretion rates of potassium are plotted

against corresponding mean concentrations in serum for all experiments, including the two with potassium sulfate previously reported (13). There is obviously

TABLE 3

*Intravenous injection of isotonic mixture of potassium bromide and potassium phosphate†*

| EXPERIMENT NUMBER | DOG WEIGHT | SALT GIVEN | PERIOD | DURATION | URINE FLOW | CONCENTRATION (M. EQ. PER LITER) | | | | | | | | CLEARANCES (CC. PER MINUTE) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Urine | | | | Serum | | | | K | Creatinine |
| | | | | | | $K^+$ | $HPO_4^{=}$‡ | $Cl^-$ | $Br^-$ | $K^+$ | $HPO_4^{=}$‡ | $Cl^-$ | $Br^-$ | | |
| | *kgm.* | *mM.* | | *minutes* | *cc. per minute* | | | | | | | | | | |
| 12 | 21.3 | | | | | | | | | 4.8 | 1.6 | 102.5 | 0.0 | | |
| | | KBr.....36.6 | 1 | 33 | 0.11 | 18 | 0 | 9 | 0 | | | | | 0 | |
| | | $K_2HPO_4$.....16.4 | 2* | 161 | 0.34 | 347 | 238 | 15 | 0 | | | | | | |
| | | | | | | | | | | 8.0 | 4.1 | | 6.8 | | |
| | | $KH_2PO_4$.....3.8 | 3 | 42 | 0.24 | 329 | 247 | 17 | 0 | | | | | 11 | 63 |
| | | | | | | | | | | 6.7 | 3.6 | 95.6 | 6.4 | | |
| | | | 4 | 58 | 0.17 | 294 | 254 | 12 | 0 | | | | | 8 | 49 |
| | | | | | | | | | | 6.8 | 3.6 | 94.6 | 6.4 | | |
| 13 | 21.0 | | | | | | | | | 4.2 | 1.8 | 100.3 | 1.8 | | |
| | | | 1 | 10 | 0.20 | 60 | 0 | 0 | 0 | | | | | 3 | |
| | | KBr.....38.5 | | | | | | | | | | | | | |
| | | $K_2HPO_4$.....17.3 | 2* | 90 | 0.92 | 267 | 135 | 37 | 1 | | | | | | |
| | | | | | | | | | | 8.2 | 4.9 | 92.5 | 9.0 | | |
| | | $KH_2PO_4$.....4.0 | 3 | 82 | 0.46 | 278 | 149 | 44 | 0 | | | | | 17 | 51 |
| | | | | | | | | | | 6.8 | 4.0 | 93.3 | 8.8 | | |
| | | Glucose.....139 | 4 | 54 | 0.35 | 275 | 170 | 26 | 0 | | | | | 15 | 55 |
| | | | | | | | | | | 6.0 | 4.3 | 93.3 | 8.8 | | |
| 14 | 30.3 | | | | | | | | | 3.5 | 1.8 | 110.5 | 0.0 | | |
| | | KBr.....77.0 | 1 | 20 | 0.10 | 170 | 0 | | 0 | | | | | 5 | |
| | | $K_2HPO_4$.....34.5 | 2* | 122 | 2.18 | 165 | 136 | | 12 | | | | | | |
| | | | | | | | | | | 8.9 | 4.7 | 91.6 | 9.9 | | |
| | | $KH_2PO_4$.....8.1 | 3 | 48 | 0.69 | 302 | 151 | | 0 | | | | | 25 | 104 |
| | | | | | | | | | | 7.6 | 3.6 | 96.7 | 8.8 | | |
| | | | 4 | 52 | 0.46 | 285 | 129 | | 0 | | | | | 18 | 81 |
| | | | | | | | | | | 7.1 | 4.0 | 96.6 | 8.3 | | |
| | | | 5 | 70 | 0.43 | 300 | 188 | | 3 | | | | | 19 | 82 |
| | | | | | | | | | | 6.6 | 4.5 | 95.0 | 9.4 | | |
| 15 | 13.4 | | | | | | | | | 4.5 | 2.5 | 107.5 | 0.0 | | |
| | | KBr.....34.6 | 1 | 20 | 0.15 | 67 | 0 | 7 | 0 | | | | | 2 | |
| | | $K_2HPO_4$.....15.5 | 2* | 113 | 0.86 | 181 | 109 | 56 | 14 | | | | | | |
| | | | | | | | | | | 8.5 | 5.8 | 90.8 | 7.8 | | |
| | | $KH_2PO_4$.....3.6 | 3 | 63 | 0.95 | 140 | 80 | 13 | 5 | | | | | 14 | 84 |
| | | | | | | | | | | 9.8 | 4.0 | 92.0 | 9.6 | | |
| | | | 4 | 83 | 0.32 | 197 | 191 | 16 | 3 | | | | | 7 | 58 |
| | | | | | | | | | | 8.0 | 3.6 | 91.5 | 7.5 | | |
| | | | 5 | 127 | 0.24 | 262 | 195 | 8 | 4 | | | | | 8 | 55 |
| | | | | | | | | | | 8.1 | 3.2 | 92.5 | 7.4 | | |
| | | | 6 | 103 | 0.19 | 326 | 246 | 5 | 5 | | | | | 9 | 52 |
| | | | | | | | | | | 6.0 | 3.1 | 95.8 | 7.5 | | |

* Indicates period during which infusion was given.

† The proportion of mono- and dihydrogen phosphates in the mixtures was such that the pH of the mixtures approximated 7.4.

‡ Millimols multiplied by 1.8.

considerable variation among the several experiments in the relationship of excretion rate to serum concentration. Nevertheless there does exist in most

individual experiments a direct relationship between excretion rate and serum concentration. The lines rise very sharply with slight increase in concentration of potassium in serum. The one exception is the line in the extreme lower right hand corner, corresponding to experiment 9 of table 2. The lines are in general not exactly straight. The upper portions, representing exogenous excretion, usually have a steeper slope than the lower portions, representing the transition between exogenous and endogenous excretion. In other words, extrapolations of the former would, in most instances, cut the abscissa at points slightly above those corresponding to endogenous excretion, and of course well above the origin. There is no definite grouping of the lines according to the salts or salt mixtures injected.

Clearances of potassium vary markedly with the concentration of potassium in serum, being greater the higher the serum concentration (tables 1, 2, 3).

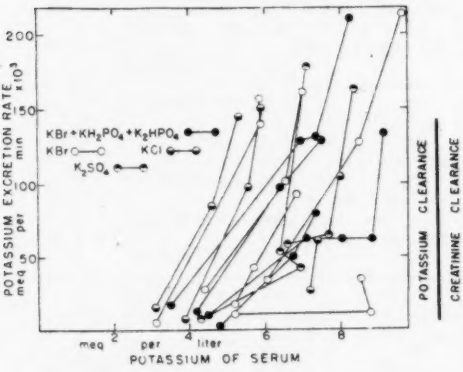


Fig. 1

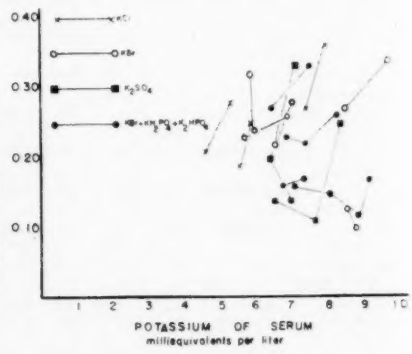


Fig. 2

**Fig. 1.** Relationship between urinary excretion rate and simultaneous concentration of potassium in serum

**Fig. 2.** Tubular reabsorption of potassium. Ratio of potassium to creatinine clearance measures the proportion of filtered potassium which escapes reabsorption by the renal tubules.

B. *Relation between potassium excretion and glomerular filtration rate.* In figure 2 the ratio of potassium to simultaneous creatinine clearance is plotted against mean serum concentration. Since the ratio of the clearances falls between the limits 0.10 and 0.40, the two clearances are fairly well correlated; in other words, clearance of potassium varies proportionally with the glomerular filtration rate, except as slight changes in tubular reabsorption perturb this simple relationship.

On the other hand, so considerable is the dispersal of the points in figure 2 that it is difficult to be sure that any correlation at all between the clearance ratio and the concentration of serum potassium exists. The difference between the clearance ratio and unity measures the proportion of filtered potassium reabsorbed by the tubules. This proportion is certainly somewhat greater during all periods of active excretion of injected potassium than during the preliminary

control periods, since a number of points lying close to the abscissa between 4 and 5 would be present if creatinine clearance had been measured in these preliminary periods. There is, however, no evidence from this chart that potassium clearance tends to approach creatinine clearance as serum concentration rises, and it is evident that, within the range of potassium which can be tolerated physiologically, the greater part of the filtered potassium is still being reabsorbed.

C. *Effect of associated anions on potassium excretion.* Sulfate is a rapidly excreted anion with a high clearance (8, 13), chloride and bromide are slowly excreted anions with low clearances (16). Phosphate in this respect lies somewhere in between (12). Nevertheless, the lines in figure 1 corresponding to these four classes of experiments are not arranged in any systematic order corresponding to the salt injected, although potassium excretion corresponding to a given serum concentration varied considerably from experiment to experiment.

The very low excretion rate of potassium in experiment 9 of table 2 (the line lies in the lower right hand corner of fig. 1) is somewhat puzzling. There was no evidence of renal disease in this animal, although creatinine clearances are somewhat low. Perhaps the explanation may be found in the fact that here the potassium bromide was injected in a hypotonic solution, and that in consequence the usual increased renal blood flow and glomerular filtration produced by an infusion of an isotonic salt solution were lacking.

D. *Relation of potassium excretion to that of other ions.* 1. *Effect on the acid base balance of urine and the excretion of bicarbonate.* A most striking effect of the injection of either potassium chloride or of potassium bromide was an immediate enormous increase in the excretion of bicarbonate as soon as the infusion was started (tables 1, 2). This was accompanied by a sharp rise in the pH of the urine, frequently to the extreme upper limits of physiologically tolerable alkalinity (fig. 3). The increased excretion of bicarbonate and the high urinary pH continued for some time after the injection was finished.

No such rise in pH of urine occurred after potassium sulfate injection (fig. 3). The pH of the urine after the infusion of phosphate mixtures was usually fixed for some time close to pH 7.4, the pH of the injected buffer itself.

2. *Effect on the excretion of sodium.* It has previously been shown that the injection of potassium sulfate is followed by a sharp increase in the excretion of sodium, which continues for some time after the end of infusion (13). In one experiment with potassium chloride there was a slight increase of sodium excretion during the infusion itself, followed at once by a fall below the pre-injection rate in the subsequent periods (table 1, expt. 3). The same is true in two of three experiments with potassium bromide (table 2, expts. 10, 11), while in a third (table 2, expt. 9) the rise of sodium excretion during injection is absent.

3. *Effects on the excretion of chloride and bromide.* The injection of potassium bromide is followed by a moderate increase in the excretion of both bromide and chloride, that of potassium chloride by an increased excretion of the latter alone. The total increased excretion of halide is much less than that of potassium.

There is no radical difference between the excretion of halide in these experiments and the excretion of halide following the injections of sodium bromide or of sodium chloride (1, 13). The ratio of chloride to bromide in urine is, as usual, higher in urine than in serum, indicating some preferential reabsorption of the bromide. Otherwise they seem to behave more or less interchangeably.

There is, however, a marked difference between the excretion of halide following the injection of potassium bromide or chloride alone and the excretion of halide following the injection of mixtures of potassium bromide with potassium phosphate. There seems to be a distinct inhibition of halide excretion in the presence of potassium phosphate. This is shown in figure 4, in which the cumulative recovery of halide following injection of pure potassium bromide and potassium chloride is compared with the recovery following injection of the mixtures with potassium phosphate.

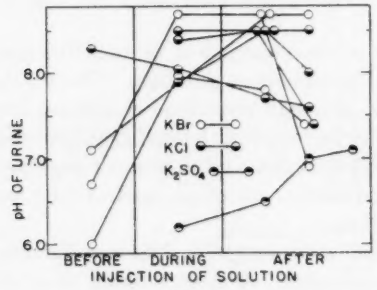


Fig. 3

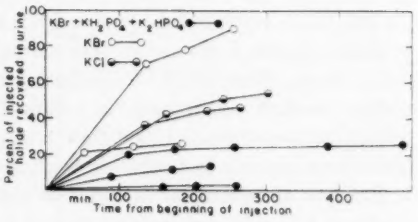


Fig. 4

Fig. 3. Effects of infusions of neutral salt solutions on pH of urine. Injection of potassium chloride or potassium bromide solution is followed by a sharp rise in the pH of urine, while injection of potassium sulfate solution is not.

Fig. 4. Effect of associated anion on the rate of excretion of injected halide. Potassium chloride and bromide are excreted more rapidly when injected alone than when accompanied by potassium phosphate.

4. *Effect on the excretion of phosphate.* No increased excretion of phosphate followed the injection of potassium chloride (table 1, exp. 3) or of potassium bromide (table 2, expts. 9, 10 and 11). Phosphate concentration in serum is elevated and urinary excretion of phosphate increased following the injection of mixtures of potassium phosphate and bromide (table 3). It is difficult to determine whether or not there is anything peculiar in its mode of excretion in these experiments, since few data on the excretion of phosphate with cations other than potassium are available.

5. *Effect on ammonia excretion.* A low rate of excretion of ammonia followed the injection of potassium chloride (table 1, expt. 3) and the injection of potassium bromide (table 2, expts. 9, 10 and 11).

Discussion. The functional relationship between the excretion rate of potassium and its concentration is clearly indicated in figure 1. The behavior of potassium in this respect resembles that of sulfate (13). The behavior of potas-

sium is similar to that of sulfate in at least two other respects. First, the lines relating serum concentration and excretion rate do not pass through the origin (fig. 1). Secondly, the straight lines representing exogenous excretion, when prolonged, do not in general pass through the points representing endogenous excretion. Because the lines do not pass through the origin, it follows mathematically that the clearance of potassium must depend upon serum concentration, being greater the higher the serum concentration. The algebraic formulation of this fact has been fully discussed in connection with the parallel formulation in the case of sulfate excretion (13), and need not be repeated here. This variation of clearance with serum concentration seems to be quite characteristic of many electrolytes. This behavior is, of course, very different from that of many organic filterable solutes, whose representative lines do pass through the origin, so that their clearances are more or less independent of serum concentration. Because the lines representing exogenous excretion do not in general pass through the points representing endogenous excretion, a curve characterizing the whole of potassium excretion cannot be represented by a single straight line. It may be approximated by two lines, one with a steep slope, representing excretion at high concentrations, and another with a lesser slope, representing excretion at low concentrations. Whether the junction of these two lines is gradual or abrupt cannot be determined from our experiments. It is certain, however, that the excretion of potassium is a different function of serum concentration after the latter has been raised by the injection of potassium salts than when it lies within the usual physiological range.

The lines of figure 1 seem to indicate that the excretion of potassium is not dependent on the associated anion. In a sense these lines characterize a specific mode of excretion peculiar to potassium. This at once brings up the fundamental dilemma which confronts the student of electrolyte excretion: how may each individual ion be excreted more or less according to its own individual law, while at the same time the sum of the acid equivalents in urine must always equal the sum of the base equivalents? Our experiments throw considerable light on this problem. When neutral potassium chloride or bromide solutions are injected, bicarbonate excretion in the urine at once increases greatly, so that the urine becomes distinctly alkaline. Bicarbonate content and pH of serum are but little affected (table 1, expt. 6). When neutral potassium sulfate is given, on the other hand, there is a marked and sustained increase in sodium excretion without appreciable change in urinary pH. Halide is characteristically excreted slowly, potassium much more rapidly; bicarbonate is apparently obligingly excreted to make up the difference. Sulfate clearance is considerably greater than that of potassium; each persists in its own stiff-necked way, and sodium is excreted in amounts just sufficient to maintain the electroneutrality of the urine. These reactions serve perfectly to permit potassium, sulfate and halide each to be excreted in its own characteristic manner. In order to do this, of course, bicarbonate and sodium are sacrificed according to the requirements of electroneutrality, rather than according to any individual laws of their own. Gamble has written elsewhere (6) of the "mendicant" rôle of bicar-

bonate in the bodily economy; perhaps we must include sodium in the same category, at least with respect to its rôle in urinary excretion under these peculiar circumstances.

The relative inhibition of halide excretion in those experiments in which potassium phosphate was injected along with potassium bromide may be in some way analogous to the inhibition of chloride excretion when mixtures of sodium chloride and sodium sulfate are injected (13). Certainly the delayed excretion is not simply a reflection of a reduced serum halide consequent upon dilution of the body fluids by the phosphate infusion, since serum halide actually rose in one of the experiments with phosphate mixtures in which halide excretion was much retarded (table 3, expt. 13). Also, in the experiment with the greatest recovery of halide following potassium bromide (table 2, expt. 7), serum halide fell during the course of the experiment.

Potassium and creatinine clearances are closely enough correlated to indicate that the former depends in part on the latter. Figure 2 gives no indication of any upper limit to reabsorption, even though the proportion of filtered potassium reabsorbed by the tubules is somewhat less after potassium injection than under ordinary circumstances. Apparently the kidney eliminates injected potassium very rapidly, partly by an increase in the amount filtered and partly by a moderate reduction in the proportion reabsorbed.

The rather high concentrations of potassium sometimes found in the urine following injections of potassium chloride or bromide are a little surprising. Concentrations greater than 400 mM per liter urine are found in experiments in which isotonic solutions were injected without subjecting the animal to any particular dehydrating procedure. In experiments with injections of hypertonic sodium chloride or sodium sulfate or in experiments with severe water deprivation the concentration of sodium in the urine seldom rises above 350 mM per liter (4, 7). It is unlikely that in such experiments the excretion of sodium is limited by osmotic factors *per se*, since higher osmolar concentrations appear in our experiments in the apparent absence of any obligation to conserve water.

## SUMMARY AND CONCLUSIONS

1. Potassium excretion in the urine following injection of potassium salts is directly correlated with serum concentration. It is little affected by the particular associated anions.

2. Potassium excretion is a somewhat different function of serum concentration at high than at low concentrations of potassium in serum.

3. Clearance of potassium varies sharply with its concentration in serum, being greater at higher serum concentrations. It varies in part with glomerular filtration rate, but never approaches the latter in absolute magnitude.

4. Intravenous injection of neutral potassium bromide or potassium chloride is followed by the excretion of an intensely alkaline urine containing large amounts of bicarbonate.

5. Intravenous injection of neutral potassium sulfate produces no change in the reaction of the urine but is followed by a marked increase in sodium excretion.

6. These experiments illustrate one type of physiological reaction which preserves the effective electroneutrality of the urine yet permits potassium, sulfate and halide each to be excreted according to its characteristic law.

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# CONTROL OF NORMAL BREATHING IN FISHES BY RECEPTORS LOCATED IN THE REGIONS OF THE GILLS AND INNERVATED BY THE IXTH AND XTH CRANIAL NERVES[1]

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In view of the fact that Heymans, Bouckaert and Dautrebande (1) and Heymans and Bouckaert (2) had found the carotid chemoreceptors sensitive to changes in the carbon dioxide tension of the blood of mammals it was suggested by Powers (3) that the regions of the gills might be the loci of receptors for chemotaxic responses of fishes to the carbon dioxide tension of the water. Instead these chemotaxic receptors were found to be located in the lateral line organs, innervated by a branch of the Xth cranial nerve (4). It was found that fishes with lateral line nerves sectioned just distal to the gills do not respond to the carbon dioxide tension of the water, even though branches of the IXth and Xth cranial nerves leading to the gills are intact. This observation eliminates the regions of the gills as loci of receptors for chemotaxic responses to the carbon dioxide tension of the water.

During this investigation (4) different combinations of sectionings of the IXth and Xth cranial nerves just central to the gills were carried out on the brook trout, *Salvelinus f. fontinalis* (Mitchell), the rainbow trout, *Salmo gairdnerii irideus* Gibbons and the blue gill, *Lepomis macrochirus* Rafinesque. Observations made seemed to substantiate suggestions made by Marshall and Rosenfeld (5) and Schmidt and Comroe (6) that the chemoreceptors "might represent the survival in the air breathing adult, of an organization of utmost significance to the water-breathing forebear." It was found that each of the three species of fishes investigated responded similarly to each combination of sectioning of the IXth and Xth cranial nerves just central to the gills (see table 1). The fishes breathed at approximately the same rate before and after the bilateral sectioning of the Xth nerves only. As a rule the fishes showed a gasping type of breathing before dying (table 1). The responses to the bilateral sectioning of the IXth nerves only were entirely different from the responses to the bilateral sectioning of the Xth nerves only. The rule without exception was the cessation of all breathing which was followed by a gasping type of breathing. When the IXth and Xth nerves were both bilaterally sectioned the responses were similar to the responses when the IXth nerves only were bilaterally sectioned except perhaps intensified. There were only two exceptions. The first two fish, brook trout, operated upon at first breathed approximately normally after the operation except at a slight reduction in rate. It is believed that portions of the IXth nerve were missed on one side. This is borne out by the fact that

[1] Contribution no. 8, Department of Zoology and Entomology, University of Tennessee, Knoxville.

breathing of all fishes was approximately normal when the IXth or Xth or IXth and Xth were sectioned on one side only. These two fishes as well as a second brook trout and a blue gill in which it was known that a part of the IXth nerve was missed are not included in the table. All four fishes responded in the same way.

When table 1 is scrutinized certain outstanding facts are noted. 1. No fish survived an operation for any length of time. Since fishes in which the gills were mutilated died almost if not as quickly as after other operations it seems reasonable to suppose that the rapid death was due to loss of blood. The efferent branchial arteries could not be avoided when the IXth and Xth nerves were sectioned just central to the gills. 2. When the Xth nerves only were bilat-

TABLE 1

The table gives a summary of breathing responses after each operation as indicated. The sectioning of the nerve or nerves indicated was just central to the gills. No fish lived longer than a few minutes after an operation. Approximately normal means that the rate of breathing was approximately the same after as before an operation.

| NO. | NAME OF FISH | TYPE OF OPERATION | KIND OF BREATHING AFTER OPERATION |
|---|---|---|---|
| 4 | Brook trout | Gills mutilated | Approximately normal |
| 3 | Rainbow trout | Gills mutilated | Approximately normal |
| 2 | Blue gills | Gills mutilated | Approximately normal |
| 6 | Brook trout | Xth bilateral | Approximately normal |
| 2 | Rainbow trout | Xth bilateral | Approximately normal |
| 4 | Brook trout | IXth bilateral | Normal breathing ceased, few gasps |
| 4 | Rainbow trout | IXth bilateral | Normal breathing ceased, few gasps |
| All | Fishes | IXth or Xth unilateral | Approximately normal |
| 4 | Brook trout | IXth and Xth unilateral | Approximately normal |
| 4 | Brook trout | IXth and Xth bilateral | Normal breathing ceased, few gasps |
| 4 | Rainbow trout | IXth and Xth bilateral | Normal breathing ceased, few gasps |
| 4 | Blue gills | IXth and Xth bilateral | Normal breathing ceased, few gasps |

erally sectioned the breathing was at first approximately normal. As a rule this was followed by a gasping type of breathing. 3. The bilateral sectioning of the IXth or the IXth and Xth nerves was always followed by a cessation of all breathing. After a few seconds, always less than a minute, this was followed by a gasping type of breathing or in a few exceptions there was a jerky type of breathing. This jerky type of breathing was interpreted as being a weak gasp. 4. When the IXth nerve or even a part of the IXth nerve leading to the gills remained intact on one side the breathing approximated normal. One rainbow trout and one blue gill, not included in the table, in which a part of the IXth nerve was known to have been missed on one side breathed approximately normally for a brief period. A gasping type of breathing was the exception (IXth nerve intact on one side) and not the rule. These observations point to the conclusion that receptors located in the regions of the gills and innervated by the IXth cranial nerves are more important in controlling normal breathing of fishes than the cephalic or central portion of the nervous system. This does not pre-

clude the localization of an "apneustic center" in the cephalic portion of the nervous system (7) but adds evidence to the views holding to the presence of an "apneustic center" and its inhibition by incoming impluses over the IXth and Xth nerves with inhibition by the IXth being more effective than by the Xth.

The apparent inhibition of the IXth might be due to asphyxiation through the loss of blood to the brain (8) but is hardly the case since the mutilation of the gills in which there was at least the same loss of blood did not produce gasping. Neither do the observations preclude the inherent rhythm of the respiratory center suggested by Adrian and Buytendijk (9) for the goldfish. The observations do add evidence to the dominance of impulses through the IXth nerves over all breathing movements. One rainbow trout in which the IXth and Xth nerves were sectioned on both sides died without breathing or gasping. This observation is evidence in support of the views held that breathing rhythm is reflexly excited. The more central the IXth nerves are sectioned the more nearly completely are all of the branches severed. Lutz and Wyman (10), Boyd (11), Herrick (12) and Pearsons (13) have described the location of receptors innervated by the IXth nerves, i.e., the innervation by the IXth nerves. Without entering into the controversy as to the origin, whether central or reflex in mammals, of impulses initiating efferent impulses from the respiratory center (14) it might be stated that the afferent reflex paths are much more restricted in fishes than in mammals due to the less high development of the brain of fishes. Fishes have no cerebral cortex. The only higher center is the rhinencephalon, mainly the nucleus of the first cranial nerves, and the corpora striata. As the brain increases in complexity, other centers as they develop might take over prominent controls of normal breathing and become less segmentally dominated (15). Summaries of current views with literature cited have been presented by Heymans and Bouckaert (2), Gesell (16), Gellhorn and Lambert (17), Pitts (18) and Schmidt and Comroe (6). Observations reported in this paper indicate that normal breathing in fishes is dominantly reflex, originating in receptors located in the regions of the gills and innervated by the IXth cranial nerves.

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## SUMMARY

1. Different combinations of sectionings of the IXth and Xth cranial nerves just central to the gills were carried out in three species of fishes.

2. The three species of fishes responded similarly to each type of sectioning.

3. When the Xth nerve was unilaterally or bilaterally sectioned breathing continued approximately normal and was often followed by a gasping type of breathing before death.

4. When the IXth or the IXth and Xth nerves were unilaterally sectioned breathing continued approximately normal. Death followed generally without gasping.

5. When the IXth or the IXth and Xth nerves were bilaterally sectioned there was always cessation of all breathing. As a rule this was followed by a gasping type of breathing.

6. From these observations it was concluded that the control of normal breathing in fishes is dominantly reflex originating in receptors located in the regions of the gills and innervated by the IXth cranial nerves.

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# THE DISTRIBUTION, FLOW, PROTEIN AND UREA CONTENT OF RENAL LYMPH[1]

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Most authors are in agreement concerning the extra-renal pathway of the lymphatics of the mammalian kidney, though there is still disagreement as to the relationship of the lymph vessels of the renal capsule and those within the kidney itself. Thus, Bartels (1), Fuchs and Popper (2) and Abeshouse (3) believe that the capsular lymphatics may participate in the drainage of the kidney parenchyma, while Parker (4) was unable to find any evidence of parenchymally injected dye in the renal capsular lymphatics. With respect to the rate of flow of lymph from the kidney, the only data are those reported by Schmidt and Hayman (5), who measured the rate of lymph flow in the thoracic duct and in the abdominal receptaculum of dogs after preliminary evisceration, unilateral nephrectomy, ligation of aorta and inferior vena cava below the remaining kidney and ligation of the portal vein and hepatic artery. The flow of lymph thus measured was considered indicative of the lymph drainage of the remaining kidney. They observed an increased lymph flow after the administration of various diuretics and of adrenalin. About the chemical composition of renal lymph, nothing is known except for a single protein determination in a sample collected directly from a dog's kidney by Drinker and Field (6).

In the present communication, the results of a study concerning the capsular and hilar lymph drainage of the dog's kidney, together with the protein and urea content of twelve different samples of lymph collected directly from the renal lymph vessels, are presented. These determinations are compared with the protein and urea content of plasma obtained from the renal artery and renal vein at the conclusion of the period of lymph collection.

METHODS. A. *Exposure of the kidney.* Normal dogs, anesthetized with nembutal (pentobarbital sodium) were employed in these experiments. A skin incision was made parallel to and one inch below the last rib on the left side and was extended from the midline to the vertebral column. Successive abdominal muscles were split in the line of their cleavage until the peritoneum was reached. The latter was incised, the posterior muscular portion of the incision bisected, and the superior and inferior flaps were then sutured to the skin of the lower external chest and lower abdomen respectively. This procedure exposed both upper and lower poles of the kidney and with a minimal amount of blunt dissection and manual traction, all surfaces of the kidney could be exposed and visualized.

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B. *Injection of Evans blue dye.* A ten per cent solution of Evans Blue dye was used for all renal parenchymal injections. A 27 gauge needle, attached to a tuberculin syringe, containing the dye, was inserted $\frac{1}{4}$ inch into the renal parenchyma for cortical injection and $\frac{3}{8}$ inch into the parenchyma for medullary injection. Approximately 0.2 to 0.5 cc. of the dye was injected slowly so as not to produce a cystic area within the kidney. A thin stream of water was directed against the injection site, as the needle was withdrawn quickly, to prevent the superficial extravasation of concentrated dye into the capsular tissue.

C. *Cannulation and collection of renal lymph.* The cannulae employed for these collections were made of glass tubing (3 mm. in diameter), one end of which was drawn out to a calibre of approximately 1 mm. in external diameter. A very small amount of dried, purified heparin was placed in the interior of the cannula to prevent clotting.

The lymph vessels of both the capsular and hilar areas of the kidney were searched for, and when visualized, ligated. Those of the capsule were ligated at each pole of the kidney, and those in the hilar area were ligated near the juxta-aortic nodes into which they drained. The preparation was allowed to stand for 20 minutes, during which time the lymph vessels proximal to the ligatures distended easily to twice their previous size. It was noted many times that ligation of the hilar vessels effected a distention in the capsular ones. A loose ligature having been placed about the distended lymph vessel, the cannula was inserted into it distal to the loose ligature and with the preliminary rapid flow of clear fluid into the cannula, the ligature was then tightened about the inserted end of the cannula. Usually 0.5 to 1.0 cc. of lymph could be collected from one of these vessels within 20–40 minutes. It was found that the flow from any single lymph trunk was accentuated by the ligation of the remaining, visible trunks. At the end of the collection, renal arterial and venous blood samples, the femoral artery pressure and the weight of the kidney of the dog were taken.

D. *The determination of the protein and urea content of the lymph and blood plasma samples.* Protein in both lymph and plasma was determined by a modification of Kingsley's biuret method (7). The color was measured in the Evelyn photoelectric colorimeter and the concentration obtained from a calibration curve based on gravimetric protein determinations. Urea was determined by a urease-aeration-titration method (8).

Results. A. *The distribution of Evans blue dye after injection into the medullary area of the kidney.* The injection of Evans Blue dye into the medullary area of 15 different kidneys of normal anesthetized dogs was followed by the appearance of the dye in the hilar lymph vessels of all injected kidneys within 0.5 to 4.5 minutes, whereas it was found in the capsular lymph vessels of only two of the 15 injected kidneys. The hilar lymph vessels were seen to traverse the loose connective tissue surrounding the renal artery and vein, emptying as has been described (4) into the lateral abdominal lymph chain. The number of vessels varied considerably in each injected kidney, usually about six large vessels being seen, some of which were in intimate association with the renal artery and vein.

B. *The distribution of Evans Blue dye after injection into the cortical area of the kidney.* Contrary to the results following medullary injection of the dye, it was found that when injections were made into the cortical area of eleven different kidneys, the dye was observed in the external capsular lymph vessels of eight of the injected kidneys, and in the hilar vessels of seven of them. In five, the dye was observed in both the hilar and the external capsular lymph vessels. The latter lymph trunks were usually two in number, each of which traversed opposite poles of the kidney to continue medially in the perirenal tissue and to end finally in the lateral abdominal lymph chain. Occasionally, a large capsular lymph vessel was observed, leaving the parenchyma of the kidney, and piercing

TABLE 1

*The flow, protein and urea content of renal lymph*

| EXPT. NO. | WEIGHT OF SINGLE KIDNEY | RATE OF FLOW FROM THE CANNULATED LYMPH TRUNK | PROTEIN CONCENTRATIONS | | | UREA CONCENTRATIONS | | | LYMPH UREA PER CENT PERCENTAGE CHANGE RELATIVE TO RENAL VEIN PLASMA UREA PER CENT | RENAL VEIN PLASMA UREA PER CENT PERCENTAGE CHANGE RELATIVE TO RENAL ARTERY PLASMA UREA PER CENT |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Renal lymph | Renal artery plasma | Renal vein plasma | Renal lymph | Renal artery plasma | Renal vein plasma | | |
| | *gm.* | *gm./min.* | *gm. per cent* | *gm. per cent* | *gm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *per cent* | *per cent* |
| 1 | 38.7 | 0.0054 | 4.21 | 5.84 | 5.92 | 84.0 | 47.5 | 44.0 | +91 | −6.4 |
| 2* | 45.7 | 0.0076 | 2.80 | 6.09 | 5.75 | 70.4 | 61.5 | 58.0 | +22 | −6.7 |
| 3a | — | 0.0102 | 3.00 | 5.35 | 5.19 | 52.1 | 22.8 | 22.8 | +128 | ±0.0 |
| 4 | 52.9 | 0.0114 | 2.35 | 5.18 | 5.18 | 47.7 | 26.4 | 24.6 | +94 | −6.8 |
| 5* | 40.4 | 0.0129 | 1.83 | 5.42 | 5.12 | 47.7 | 38.7 | 35.2 | +36 | −9.0 |
| 6 | 44.2 | 0.0153 | 2.04 | 5.55 | 6.35 | 48.4 | 44.0 | 44.0 | +10 | ±0.0 |
| 7 | 56.6 | 0.0229 | 0.96 | 5.60 | 5.57 | 58.6 | 52.8 | 51.0 | +15 | −1.5 |
| 8* | 40.3 | 0.0270 | 1.12 | 6.88 | 6.52 | 112.5 | 82.6 | 79.1 | +42 | −4.2 |
| 9 | 64.5 | 0.0329 | 0.44 | 6.45 | 6.45 | 164.0 | 154.6 | 151.2 | +9 | −1.2 |
| 3b | — | 0.0352 | 1.41 | 5.35 | 5.19 | 42.2 | 22.8 | 22.8 | +85 | ±0.0 |
| 10 | — | 0.0425 | 0.56 | 6.10 | 6.60 | | | | | |
| 11 | 43.1 | 0.0550 | 1.34 | 5.95 | 5.81 | 38.7 | 30.8 | 29.9 | +33 | −3.8 |
| Average..... | | 0.0232 | 1.84 | 5.81 | 5.80 | 69.7 | 53.1 | 51.1 | +55 | −3.6 |

* Renal lymph obtained from capsular vessel.

the fibrous capsule to join one of the two large trunks running in the fatty capsule toward either pole. Invariably, ligation of the external capsular lymph trunk was followed by distention of the trunk segment proximal to the ligature, indicating that the flow of lymph was distalward from the kidney.

C. *The distribution of dye following intra-renal artery injection of Evans Blue dye.* Injection of the dye (2.0 cc.) directly into the renal artery of three different kidneys was followed with 0.5 to 1.0 minute by the appearance of the dye in both the external capsular and hilar lymph vessels of each kidney. It was found also that whereas the parenchyma of the kidney (both cortical and medullary areas) was rapidly and uniformly stained with the dye, the perirenal tissue beneath the capsule was slightly or not at all colored despite the fact that its lymph trunks were seen to be deeply colored by the injected dye.

D. *The rate of renal lymph flow.* The total renal lymph flow rate cannot, of course, be obtained from the observed rates of flows of single lymph vessels (table 1), but a maximal approximation, valid for our conditions of observation, may be made. For in no dissection of perirenal tissue following the injection of dye, were there more than ten visible lymph trunks leaving the kidney. Thus a maximal rate of lymph flow may be calculated by multiplying the maximal total number of lymph trunks (ten) by the highest recorded lymph flow (0.0550 gram/min., table 1). This calculation would give the figure 0.5 gram per minute as the maximal total flow for a single kidney. Admittedly, our conditions are so unphysiological that we cannot attach any general significance to this figure, but it is of interest to observe that this rate of 0.5 gram per minute is only about 2 per cent of the total rate of fluid reabsorbtion as calculated from estimates of the glomerular filtrate rate and the rate of urine excretion.

E. *The protein content of renal lymph.* Twelve samples of renal lymph (nine hilar, three external capsular) were collected from eleven kidneys by direct cannulation of a lymphatic vessel leaving the kidney. The average protein content of the twelve samples equalled 1.84 grams per cent, although the individual concentrations of protein varied from 0.44 to 4.21 grams per cent (table 1) and appeared to vary inversely with the rate of flow. For example, in experiment 3a and 3b, the same kidney was cannulated twice. The first lymph flow occurred at a rate of 0.01 mgm. per minute and the lymph had a protein content of 3.0 grams per cent, whereas the second collection from a different vessel gave a lymph flow of 0.035 mgm. per minute with the protein content of 1.41 grams per cent. No striking difference was observed between the protein concentration of external capsular and hilar lymph. It is worthy of note that whenever the rate of flow is not smaller than usual, the protein concentration in renal lymph is lower than the concentration found in lymph from the thoracic duct, liver, lung and heart (6).

F. *The urea content of renal lymph.* Urea determinations were performed upon eleven lymph samples obtained from ten different kidneys. In table 1 it is shown that the urea concentration in the lymph is always higher than the urea concentration in either the arterial or the venous plasma. Special experiments on composite lymph samples showed that the excess "urea" was not ammonia and that it did not arise because heparin was used to prevent lymph clotting, whereas potassium oxalate was employed as an anti-coagulant for the blood. The second last column in table 1 indicates how considerable and yet how variable is the increase in lymph urea concentration relative to that observed in the plasma from the renal vein. The last column gives the reduction in the urea concentration of the venous plasma relative to the concentration in the arterial. This may be taken as a rough index of the urea excretory activity of the kidney, for it has been shown that when the formation of urine ceases this relation is reversed and the vein comes to contain a greater concentration than the artery (9). There is no instance of such a reversal in these observations, though there are three cases in which no difference between vein and artery could be measured. However, there is no constant relation between the increase in lymph urea concentration and the decrease in venous, relative to arterial urea concentrations,

and though the differences in the times of collection of lymph and blood make it impossible for us to attach much significance to this negative fact, the increase in lymph urea is so pronounced as to render it very improbable that variation in renal activity can be the only or the main factor involved. It seems to us that the attempt to explain this interesting fact should be deferred until further experimental evidence has been obtained.

SUMMARY

1. Lymph was collected by cannulation of both capsular and hilar lymphatic trunks. Experiments with dye injections into the renal artery, the cortex, and the medulla indicated that the flow was towards the capsular lymphatics from the cortex and toward the hilar lymphatics from the medulla.

2. The concentration of protein in the renal lymph varied from 0.4 to over 4.0 grams per 100 cc. The slower the lymph flow the greater in general was the protein concentration.

3. The concentration of urea in the renal lymph was always greater, often considerably greater, than the concentration of urea found in the plasma of the renal artery or vein.

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# THE LOCUS AND THE NATURE OF THE A-V PAUSE IN THE SPREAD OF CARDIAC ACTIVATION

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*Introduction.* One of the inadequately explained phenomena manifested by the vertebrate heart is the junctional delay in the spread of activation. The pause at the atrio-ventricular junction of the mammalian heart has received particular attention in its relation to clinical disturbances of conduction. For the dog heart Hering (1910) concluded that much of the atrio-ventricular delay occurs during the passage of the wave of activation through the node of Tawara. The nature of the process responsible for this delay has, however, remained obscure. Attempts to explain the A-V delay by application of known facts concerning cardiac excitation and conduction have made use of two general types of theory. One involves a concept of latency of ventricular response to the atrial impulse as a stimulus. The other conceives of a delay due to slow transmission through junctional tissue. The possibility that a considerable part of the A-V delay might be attributable to a latency occurring at some point of transmission from one type of tissue to another was suggested by Erlanger (1912). Earlier measurements such as those of Trendelenburg (1911) showed a significant but brief delay between the application of an induction shock and the earliest recorded response but neither these nor later experiments have demonstrated a true latency of response to brief shocks which is long enough to account for the observed junctional delays. Consequently, although arguments have been presented in support of one or another form of "latency" theory they have not met with general acceptance.

In contrast to the latency type of theory are the "transmission" theories which, in logic, have retained essentially the interpretation developed by Gaskell (1900) who postulated that a slower conducting power of the tissues between auricles and ventricles resulted in the observed pause between auricular and ventricular contractions. Ashman (1930) discussed the evidence for the two types of theory and concluded that acceptance of the "latency" theory was unnecessary either for the turtle heart or for the human heart.

It is the purpose of this paper to present material which we believe indicates the possibility of applying a type of "latency" theory, or better, an "excitation time" theory of atrio-ventricular delay to the explanation of this phenomenon. The experimental material deals with electrical excitation of the ventricle and with conduction between the atria and the ventricle, or the reverse, in the heart of the turtle. For most of the experiments we have used the slider terrapin (*P. elegans*). In the turtle the atrio-ventricular connection has been described as a simple muscular junction (Laurens, 1915), the atrial fibers coming into contact with the ventricular fibers. At a room temperature near 25°C. the

normal A-V delay is about 0.4 second. As a basis for a theoretical interpretation we shall make use of the generally accepted assumption that, for the normally conducted impulse, activation of ventricular tissue is effected by the action potential of the atrium.

*Response Times to Stimulation by Electric Currents.* a. *Procedure.* If an "excitation time" theory is to account in any considerable part for the normal range of observed variations in A-V delay it must be shown that the ventricular tissue is capable of responding to applied electric currents after correspondingly long periods of current flow. That is, the minimal demonstrable range of excitation times to applied currents should amount to several tenths of a second. Such excitation times would be impossible if the ventricle developed severe accommodation to applied electric currents or if the ventricle showed only the vanishingly small latencies to currents from auricular tissue indicated by earlier work in which induction shocks were employed as the stimulus to the ventricle. Gilson and Peugnet (1932) reported experiments on turtle ventricular strips which indicated that these did show a considerable degree of accommodation to constant currents. In those experiments the strips were routinely prepared and arranged on electrodes in a moist chamber and preliminary adjustments of switches and other devices were made. This required the passage of fifteen minutes or more and during that time a sufficiently stable condition of excitability was reached so that systematic readings could then be begun. Despite the injury involved in cutting the strips, the absence of perfusion and the repeated elicitation of responses, occasional preparations showed little if any accommodation.

Since that time we have repeatedly observed that the turtle ventricle perfused with suitable oxygenated, buffered saline solution can be maintained for many hours with little sign of deterioration of excitability and that such a preparation, when thoroughly recovered from a previous response, may display but slight accommodation to applied electric currents. In most cases the preparations used in this investigation of excitation times have been maintained by a Straub type cannula, the saline mixture in the cannula being buffered with sodium bicarbonate and bubbled with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. In arranging a preparation, the entire heart was excised, the cannula tip was inserted through the aorta into the ventricle and tied. A ligature was then drawn around the atrial and funnel tissue and tied tightly to prevent conduction from atria to ventricle. With turtles from the laboratory stock, this usually resulted in a quiescent preparation. The material discussed in this section refers exclusively to ventricular preparations in which there were no spontaneous rhythms, or to preparations in which beats appeared at such infrequent times that stimuli could be applied at 30 second intervals with only occasional complications due to spontaneous contraction. The excitability of such a preparation is probably below that of a normal intact and active animal. Stimulating currents were applied through calomel electrodes which showed no significant polarization with test currents (ca. 800 microamperes) much higher than those used for near threshold stimulation (usually 50 to 200 microamperes). Contact with the heart was made by means of a saline soaked bit of yarn which

was placed against the moist heart but was not otherwise attached to it. Amplifier leads were placed intimately about the wick of the cathodal stimulating electrode. In later experiments the cathodal stimulating electrode served as one of the grid lead electrodes constituting one amplifier lead which thus recorded from the point of first response in the heart. The positions of the other two leads were adjusted to give a conveniently small stimulus artefact on the electrographic record. It was sometimes possible to lead directly to the grids of a three-stage differential amplifier coupled with 2 mfd. condensers and 1 megohm grid leak. For the records used for reproduction below, input to the grids of the first amplifier stage was through 0.01 mfd. condensers with half megohm grid leaks.

b. *Constant currents.* Experiments were first carried out using constant currents. Excitation times of 0.4 to 0.7 second were repeatedly recorded. In a few instances significantly longer latencies were recorded. However, to obtain such long time values it was necessary to use current strengths so close to threshold as to make reproducibility of response extremely uncertain. Since a normally beating and conducting heart seems to possess a reasonable margin of safety, and since the cardiac action potentials do not have the form of constant currents, no extensive effort was made to pursue this line of attack.

c. *S-shaped currents.* S-shaped currents were obtained by discharging a condenser connected between grid and cathode of a type 89 tube used as a triode. By use of a variable resistance as grid leak the rate of drainage could be controlled at will. The resulting plate current plotted a curve which rose from cut-off, passed through an early inflection and, for the upper 80 per cent of its course, closely approximated a simple exponential. With such currents, response times of 20 seconds or more have repeatedly appeared and the results are quite reproducible. There is striking similarity of appearance between many of our records and those recently published by Skoglund (1942) for nerve. Obviously such current forms do not reproduce those of the cardiac action potentials. The experiments have, however, demonstrated clearly that the excitation time for response to electrical stimulating currents may be of almost indefinite duration provided that one uses currents of proper form. Considered from a somewhat different aspect, it is seen that the limiting slope for successful excitation of heart muscle may be very slight.

d. *Double condenser shocks.* To achieve a stimulus form more closely resembling that which might be expected of an action potential, somewhat distorted double condenser shocks were obtained by connecting a bridge stimulator (Monnier, 1934) between grid and cathode of a type 89 tube, used as a triode. For shock forms having a rising time measured in seconds or tenths of seconds and with current strengths adjusted to be close to threshold value, the ventricular response occurred at the crest of the wave or before any considerable fall-off had occurred. With shock strengths, increasingly above threshold, the response occurred at earlier times, during the rising phase of the stimulus wave (fig. 1). These results were confirmed by approximate graphic construction using the subtangent method of Rushton (1937).

*Locus and relative duration of the junctional delays.* a. *Use of electrograms*

*obtained by differential leads.* To determine the precise locus of the delay which occurs in the transmission of activation across the atrio-ventricular junction, arrangements were made to obtain records from points as close as possible to the actual junction. Using excised turtle hearts the ventral surfaces of atria and ventricle were removed by cutting with fine pointed scissors. The preparation could thus be opened out on wax so as to expose the inner dorsal surface of atria and ventricle. Close to the A-V junction the connection was cut to leave a conducting bridge which usually consisted of the right portion of the dorsal wall and which was from two to four millimeters in width. Two pairs of needle electrodes, each with a needle separation of about one millimeter, were placed near the junction and were so supported that one pair or the other could be moved for exploration. Although attempts were made to avoid injury by pressure many of the records show indications of some injury effects under one

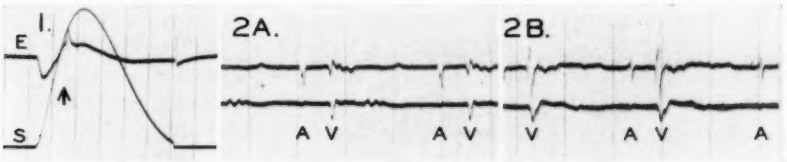


Fig. 1. Excitation time of 1.2 sec. to somewhat distorted double condenser stimulus of greater than threshold intensity. Moment of response indicated by arrow. Perfused turtle ventricle. Time intervals of 1 sec.

Fig. 2. Records obtained by pairs of needle electrodes, each pair having a needle separation of approximately 1 mm. Opened heart with conducting bridge 3.5 mm. wide. Wet preparation with consequent large "extrinsic" lead effects. Time intervals of 1 sec.

A. Electrode pairs separated by 6 mm. Leads for upper record were placed on the atrial side, for the lower record on the ventricular side of the A-V junction. Apparent pause is 0.63 sec.

B. Upper electrode pair was moved downward to within 1 mm. of lower pair, being on the ventricular side of the A-V junction. Apparent A-V pause of 0.6 sec. Records with intermediate electrode positions showed the same A-V pause.

of the needles Leads from each pair of electrodes were connected to the grids of a differential amplifier which activated a recording galvanometer. A common ground lead was placed, somewhat removed from the heart, on the moist surface of the wax. It was thus possible to place electrodes to touch the heart at desired points and at distances as little as one millimeter from the actual junction. In no experiment in which the preparation showed a normal A-V delay was there found any intermediate junctional region which indicated a significant slowing of conduction. The preparations showed rapid conduction through atrium or through ventricle but an interval essentially that of the full A-V pause occurred between activation of those parts of atrium and ventricle adjacent to the junction (fig. 2).

b. *Effects of stimuli applied above and below the junction.* That normal junctional delay occurs at a sharply localized boundary was indicated even more strikingly by the results of stimulation experiments. When electrodes were

applied to various points on the outside of the intact, perfused heart, close to the atrio-ventricular boundary, stimuli might elicit premature responses of atria and ventricle simultaneously, of atria followed after a junctional pause by ventricle or of ventricle followed after a junctional pause by a response of the atria. At certain times in the heart cycle the conducted responses of ventricle or atrium would not appear but in no case did there appear any junctional pause of intermediate duration as might have been expected under the "transmission" hypothesis which assumes the existence of a tissue having a "slowed conducting

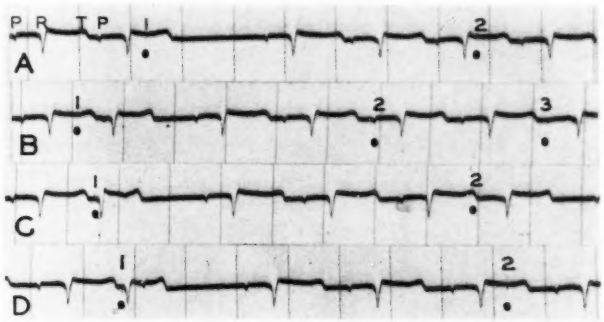


Fig. 3. Effects of position of stimulating electrodes on the response times of the different chambers of the heart. Excised heart, opened ventrally and with A-V connection reduced to 2 mm. bridge. Silver wire stimulating electrodes with 3.5 mm. separation, placed axially and close to the A-V junction. Time of application of stimuli indicated by dots. Heart beating spontaneously and with apparently normal conduction. Time intervals of 1 sec.

A and B. Initial ventricular complex indicated by R, final ventricular complex by T and initial atrial complex by P. Cathodal stimulating electrode toward atrium. Properly timed stimuli caused response of the atrium with (B-1) or without (A-1) conduction to and response of the ventricle. There were no direct responses of the ventricle to the stimulation.

C. Electrode position same as for A and B but polarity was reversed, the cathodal stimulating electrode being toward the ventricle. There were simultaneous responses of both atrium and ventricle (C-1) or of atrium alone (during refractoriness of the ventricle) followed by conduction to and response of the ventricle (C-2).

D. Electrodes moved 1.5 to 2 mm. toward ventricle. Polarity unchanged from that used for C. Atrium was not affected by stimulus. Ventricle responded if not refractory.

The apparent A-V delay is about 0.5 sec. in all cases, no significantly intermediate times having been found in any records.

power". In an attempt to place electrodes more precisely, we again made use of the excised heart pinned out on wax, opened ventrally and with the A-V junction cut down to a narrow conducting bridge. Shocks of brief duration were applied through silver wire electrodes. Results from such an experiment are indicated by the records of figure 3. For record strips A and B, stimulating electrodes having tips separated by 3.5 mm. were placed axially along a 2 mm. conducting bridge and close to the A-V junction. The cathodal electrode was toward the atrium. Atrial premature responses were elicited (fig. 3, responses A-1, B-1) and if the stimuli were properly timed (B-1) there was conduction

across the junction with consequent ventricular response. Direct stimulation of the ventricle did not occur with this electrode position. For strip C, the positions of the stimulating electrodes were unchanged but their polarity was reversed, the cathodal wire being that closer to the ventricle. Stimulation now caused simultaneous responses of atrium and ventricle (C-1), of atrium followed by conduction to and response of the ventricle (C-2) or of ventricle alone according to the excitabilities of the respective chambers at the moment of stimulation. Without change of electrode polarity (cathode still toward ventricle) the electrodes were moved about 2 mm. toward the ventricle and stimulation produced responses only of the ventricle (D-1) never of the atrium. The electrodes were repeatedly passed back and forth across the junction and the results were invariable. If only one chamber (atrium or ventricle) responded to the stimulus directly, there might be conduction to the other chamber but if this occurred it required the full expected time of normal junctional delay. There were never any records showing intermediate shorter times of delay such as would be expected under the "transmission" type of theory. The fact that one could stimulate atrium during the absolutely refractory phase of the ventricle (B-1) and still have conduction across the junction to the ventricle with ventricular activation offers further food for thought in this connection.

Individually and collectively the records of differential lead electrograms and the stimulation experiments offer strong evidence that the junctional delay in transmission between atrium and ventricle occurs at a sharply delimited locus. It is presumed that this locus is identical with that of the anatomical A-V junction. This conclusion is further strengthened by visual observation of active preparations studied under the dissecting miscroscope. Particularly striking was the sort of observation made when there was more or less independence of atrial and ventricular rhythms. In one case, for example, there occurred a brief period of ventricular fibrillation during which there was complete or nearly complete A-V dissociation. Contraction of atrial fibers could be seen on the one side and of ventricular fibers on the other side of the junction. The intermediate region of extinction involved at most a small fraction of a millimeter.

*Comparison of Junctional Delays Occurring with Normally Directed and with Retrograde Conduction.* Experiments were performed to investigate the relationships existing between junctional delays for normally conducted and for retrograde impulses. Using a perfused, excised heart, lead electrodes were placed on atrium and ventricle and a pair of stimulating electrodes were placed on the ventricle. The heart was allowed to beat spontaneously with normally directed, A-V conduction and then, using a brief condenser discharge as stimulus, the ventricle was stimulated at a rate slightly faster than that of the normal rhythm. With proper phase relations between stimulus and the beat of the normal pacemaker, ventricular beats were conducted in a retrograde direction and caused responses of the atria. One record obtained by this method showed the A-V interval to be 0.44 sec. The V-A interval was 0.73 sec. Such times are typical although in some preparations the A-V and V-A delays may approach even one another more closely.

The record of another example of retrograde conduction is shown in figure 4. An atropinized heart was pinned out on wax and opened ventrally, the A-V junction was reduced to a 2 mm. bridge and stimulating electrodes were so placed that the cathode was about 2 mm. on the ventricular side of the A-V junction. The heart continued to beat spontaneously and the A-V delay was 0.48. When the ventricle was stimulated so as to cause retrograde conduction the V-A delay was about 0.9 sec. Some variation due to fatigue, etc., is indicated. Coming after the initial atrial deflection (P-wave) which follows the ventricular response numbered 5 there may be seen a slight deflection equivalent to an atrial T-wave. The normal A-V pause is such that ventricular activation first occurs during the atrial T-wave. Furthermore it is to be seen that where retrograde conduction occurs (fig. 4, following ventricular responses 2, 4 and 5) there is beginning activation of the atrium during the ventricular T-wave. With due allowance for conduction times the time of the crest of the T-wave corresponds to the moment of most rapid fall of a monophasic action potential and it

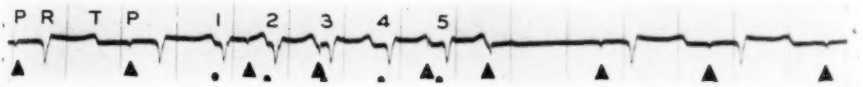


Fig. 4. Retrograde conduction recorded from same preparation as that used for records of figure 3. Cathodal stimulating electrode on ventricular side of the junction. Times of application of stimuli indicated by dots. Responses of atria indicated by triangles.

Shock 1 caused premature response of ventricle, no retrograde conduction.

Shock 2 caused response of ventricle with retrograde conduction to and response of the atrium.

Shock 3 stimulated the ventricle but produced no apparent effect on the atrium.

Shocks 4 and 5 stimulated the ventricle and there was retrograde conduction to the atria in each case. The atrial response following response 5 of the ventricle shows a barely perceptible atrial T-wave.

will be seen in the theoretical section below that this is approximately the time at which transjunctional stimulation would be expected. In a large number of records taken at room temperature and showing clear atrial or ventricular T-waves this relationship has held without exception.

Figure 5 represents in schematized form the condition which may exist at a small region of the atrio-ventricular junction. It is of fundamental importance to the interpretation of the diagram that the ventricular element be considered as completely external and extracellular to the atrial element. Cellular continuity and relatively rapid, continuous conduction may exist throughout atria or throughout ventricle but is believed to be effectively interrupted at the atrio-ventricular junction. Elements of atrium (A-C) and of ventricle (C-D) are diagrammed as lying on either side of a simple atrio-ventricular junction (C) and to be lying in a plane conducting medium. The atrium may be considered to be in one of two arbitrary conditions: 1, with the advancing wave of atrial *excitation* at a point half way along the diagrammed atrial element, or 2, with the advancing front of *recovery* half way along the atrial element. It is recognized that both the times selected and the sharpness of demarcation at the boundary

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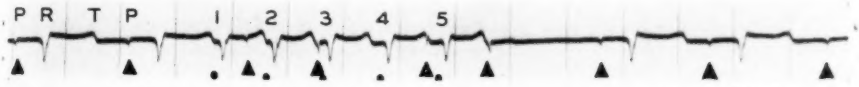


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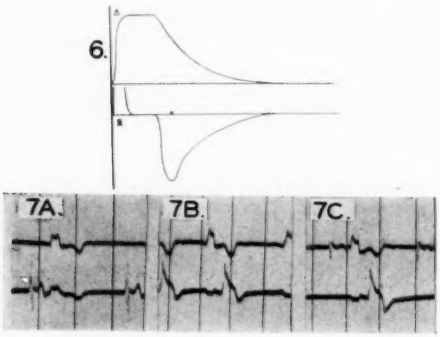
B are arbitrary and they have been chosen only for convenience in diagramming and description. In more general terms, the first condition may be regarded as occurring at any moment during the rise and spread of electrical activity



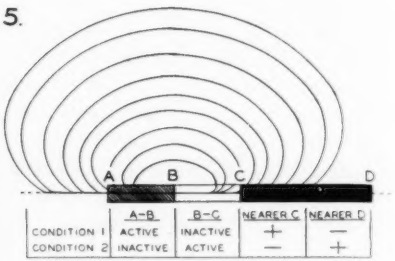




Fig. 5. Diagram indicating the direction of external electrotonic current lines (drawn only for the upper half of a schematic plane conducting field) resulting from the atrial action potential during times for:

Condition 1, when the atrial element (A-C) is relatively active at the left (A-B) and still relatively inactive at the right (B-C); and

Condition 2, when the atrial element is relatively recovered at the left (A-B) and is still relatively active at the right (B-C).

The adjacent ventricular element (C-D) is considered to be completely external to the cell structure comprising the atrial element and to be in a resting state for both conditions of the diagram.

It will be seen that during the arrival and development of the atrial action potential at such a junction of abutment (at C), there is local relative positivity of that part of the surface of the ventricle near C (condition 1). During the period of electrical recovery which roughly coincides with the fall of the monophasic action potential of the atrium (condition 2) there is local relative negativity of that part of the surface of the ventricular element near C and ventricular excitation would be expected to occur during this period.

Fig. 6. Curve A. Schematized diagram of cardiac (atrial) monophasic action potential.

Curve B. Plot of differential curve of A. If the second (downward) phase of B represented the plot of a stimulating current of just threshold value, response of the stimulated (ventricular) element would be expected at or very shortly after the moment indicated by the black dot.

Fig. 7. Records from depressed preparation which displayed long apparent A-V pause to show intermediate response of tissue near A-V junction occurring between responses of atrium and of ventricle. Paired silver wire lead electrodes were placed to touch lightly against the preparation.

A. Leads from right atrium (lower record) and from intermediate region at A-V junction (upper record).

B. Leads from junctional region (upper record) and from points near the middle of the anterior ventricular surface (lower record).

C. Leads from atrium (upper record) and from ventricle (lower record).

through the atrium. The second condition may in its general sense represent the situation at any time during the recovery of the atrium toward the electrical resting state. The atrial element is considered as being first activated at the

left end of the diagram. Schematic lines indicating flow of electrical current have been drawn only for the upper half of the plane figure.

For condition 1, representing a time during the activation of the atrium, the region at the left of B is negative to the region at the right of B. The orientation of potential differences along the ventricular surface under this condition is not such as to result in ventricular stimulation at C during the arrival or rise of the action potential at the A-V junction. The condition which might apply at an instant when complete activation of the entire atrium has been achieved and the entire atrial surface has reached an equi-potential state, if it exists at all, is very transient. During this time no current will flow from one part of the atrium to another. Consequently no electrotonic currents will be set up along the ventricle. No potential differences will be established along the surface of the resting ventricle and no stimulating effect will be manifested during this period. A third possibility is indicated by the second condition of figure 5. The electrotonic currents set up across the recovering-active boundary of the atrium will establish potential differences along the ventricular element which will be so oriented that the ventricular region nearer C will be negative to the region nearer D. Consequently, if these potential differences were developed with proper time and intensity functions, transjunctional stimulation of the ventricle would be expected to occur.

It is evident that the potential changes which are established along the ventricular surface are affected by the form of the atrial action potential. Thus, disregarding conduction, an approximation to the curve of potential which results in stimulation of the ventricle can be had by plotting the differential curve of the monophasic atrial action potential, i.e., the curve of potential differences, at successive times, between the terminal region of the atrial element and a second region on the atrium just to the left of it, or, and more properly, a curve of the slopes, at successive times, of the monophasic action potential. Such a pair of curves have been diagrammed as figure 6. Curve A represents a highly schematized monophasic action potential. Curve B plots the differential curve of A. The vertical ruled line indicates zero time for the two curves. For a monophasic curve of the form plotted, and with an intensity of atrial action potential such as to be barely above threshold for ventricular stimulation, ventricular response would be expected to occur at, or very shortly after, the time indicated by the black dot (see section dealing with double condenser stimuli). The local atrio-ventricular delay would therefore be approximately the time from the zero time line to the dot. An atrial action potential of greater than threshold value would stimulate at a time earlier than that indicated by the dot but not at a time earlier than the beginning of the downward phase of curve B. An increase of steepness of the recovery phase of the monophasic curve would, other things remaining constant, be expected to give a shortened A-V delay. A flattening of this curve would correspondingly be expected to increase the A-V delay. Lengthening or shortening of the duration of the monophasic action potential would likewise, other things remaining constant, be expected to lengthen or shorten the A-V pause. Such variables can be predicted from

experiments with double condenser stimuli and the range of excitation times found in those experiments are more than adequte to account for the normal time range of observed junctional delays.

*A-V Conduction with Long Delay.* The material presented above does not take into account the long A-V delays which are often seen in the laboratory. At temperatures of about 25°C. such delays are frequently about 0.8 sec. in duration but may be longer. In attempts to obtain preparations showing such long times between responses of atria and ventricle several procedures were tried, one of the most satisfactory being as follows. The atropinized heart was excised and removed to a shallow dish containing a thin layer of saline. The ventral opening of atria and ventricle was carried out as described above and a sagittal or parasagittal cut was then made through the ventricle and into the atria. The ventricle was thus completely divided into two parts both of which were activated by impulses which arrived over more or less normal paths from the sinus pacemaker. If necessary, further embarrassment of A-V conduction was accomplished by reducing the width of the conducting bridges between atria and ventricle. After the sagittal cut had been made one part of the ventricle, usually the left, generally remained in standstill for some minutes and then resumed a conducted response, frequently with partial block and frequently with a significant lengthening of the A-V pause. It became evident that the long A-V times which were recorded in these preparations either were or closely approached being simple multiples of the shortest A-V time which was actually observed or which might have been expected as normal on a basis of previous experience. That is, using a hypothetical example, if the A-V time of the freshly exposed heart was 0.4 sec., the A-V pause for the right side of such a maltreated preparation might be 0.8 sec. and that for the left side might be 1.2 sec. If premature stimuli were applied to the atria, conduction to the right ventricular portion might require 1.2 or 1.6 sec., and conduction to the left ventricular portion might require 1.6 or 2.0 sec.

Such results presented but two simple alternatives. Either the conclusions drawn from the preceding sections were untenable or additional finite increments of delay had entered the system. The latter proved to be the case. An opened, cut heart from which records had been obtained was studied under the dissecting microscope. Between atria and left ventricle two other serially activated, discrete responses could be seen to occur within a region extending 1.5 to 2 mm. from the A-V junction. Partial blocks existed between each of these four increments so that at one counting the rhythms were 2:1, 3:1, and 2:1, respectively. Thus, for the period considered, there were 12 beats of the atrium to 1 beat of the ventricle. The electrical records which had been made a few minutes previously showed an A-V delay 4 times the normal, probably indicating either that one responding increment was not seen or that there had been a change in the number of functional mechanisms responding. For the right part of the same preparation, electrical records had indicated a delay 3 times the normal. One intermediate responding element was clearly observed.

Electrical records were made from such preparations, and, if electrodes were

carefully placed, the recorded electrograms showed responses occurring at times predictable as multiples of the normal junctional delay for the freshly exposed heart. In most cases movement of the electrode by a millimeter was sufficient to bring in or completely lose the recorded response of the small intermediate increments. Occasionally a considerably larger part of the endocardial surface of the ventricle was observed to show movement, an observation first made in the case of a preparation which displayed partial block between this region and the rest of the ventricular mass. Figure 7 presents records from a preparation showing such an extensive intermediate region. A response was recorded by electrodes placed on the ventricular endocardium or the ventricular ring at a time roughly midway between the times of responses of atrium and of ventricle. The laboratory temperature was 32°C. at the time and other records taken from the preparation indicate that the delay times apparent in these records (about 0.6 sec.) are twice the duration of the unit delay time for the preparation. In most cases the time functions recorded indicated that the intermediate responses have properties of atrial rather than of ventricular tissue. A few ambiguous cases may involve delays longer than this and may be concerned with delays of truly junctional type appearing between increments of ventricular tissue. Study of microscopic sections cut from one preparation which had been carefully observed in action indicated that the most critical region for the appearance of delays intermediate between atrium and ventricle is that in which atrial fibers are entering the ventricle and establishing contact with the muscular ring at the ventricular base.

Discussion. The repeated observation that long A-V delays are associated with the introduction of one or more visibly and electrically responding increments interpolated between atrium and ventricle makes it possible to explain such long delays under the hypothesis presented above. The observation also offers a possible explanation for the very long A-V times which have been reported in the clinical literature (see Barker and Bridgman, 1917; Herrmann and Ashman, 1926) and which have been difficult to explain under the classical theories of junctional delay.

A clinically recognized phenomenon representing the opposite extreme from the very long A-V times is that of the short P-R interval found with the syndrome of Wolff, Parkinson and White (1930). The short P-R interval has been interpreted by Wolferth and Wood (1933) as possibly due to the presence of an aberrant or accessory atrio-ventricular conducting bundle. Recently Butterworth and Poindexter (1942) have reported experiments which they considered as confirmatory of this interpretation. The latter workers used the amplified action potential led from the dog or cat atrium as a stimulus to activate the ventricle. The resulting electrocardiograms showed great similarity to those recorded clinically from individuals showing the Wolff-Parkinson-White syndrome. In the light of the material which we have reported above, it appears that the highly significant aspect of the experimental results of Butterworth and Poindexter lies not in the introduction of an aberrant pathway to cause stimulation of the ventricle but rather in the fact that the accessory path was

such that stimulation of the ventricle occurred without the usual junctional delay. It seems probable that if a few conducting fibers in the heart of occasional humans were to develop anatomical relationships or excitability characteristics such as to make possible an effective short-circuiting of the usual truly junctional connections in the A-V node, there would be precisely the shortening of P-R interval which is actually seen. A reverse phenomenon would result in abnormally long A-V delays. The abnormality then would be one concerned with delicate anatomical or excitability relationships between connecting cardiac fibers rather than one involving the presence of actual accessory pathways for conduction. The belief that such may be the case is heightened by the variety of disturbances in rhythm which may be developed experimentally in the heart by use of a technic similar to that described by Butterworth and Poindexter. These disturbances closely simulate conditions which have been recorded clinically.

The experimental results which we have obtained indicate that in the turtle conduction from atrium to ventricle, as observed normally, depends upon the use of a path involving a single junctional delay. That this path may not always be the same anatomically is indicated by the conflicting results found in the earlier literature on the subject. In our experience there have been instances of profound and even permanent A-V block resulting from small incisions into the right lateral part of the A-V connection. On the other hand a small injury into the left region has on two occasions proved equally serious, the right side which remained intact conducting only infrequently and with long A-V delay. Changes in heart rate will cause minor changes at the normal, single junction which may, of course, involve the activity of extremely few fibers. In the depressed, prematurely activated preparation spread of excitation by this path involving only the single junctional delay may fail. Paths involving the use of two or more junctions may then come into play and the measured A-V interval will show a corresponding multiplication of the apparent delay. It seems not impossible that transjunctional conduction in other types of heart or in other tissue complexes may involve functional mechanisms similar to those which we have studied.

SUMMARY. 1. We have reported above upon experiments which were performed in an attempt to determine the locus of the atrio-ventricular delay in the heart of the turtle and to determine the cause or nature of this delay in transjunctional conduction.

2. Electrical currents of several forms have been used in stimulation of excised perfused ventricles. Slowly rising S-shaped currents or currents approaching double condenser waves in form were found to give excitation times which could be many seconds in duration.

3. Electrical records of normally conducted impulses, results of experiments with electrical stimulation eliciting premature responses and visual observation all lead to the conclusion that the normal A-V delay occurs *at* the locus of the atrio-ventricular junction.

4. During normal A-V conduction, the beginning of ventricular electrical activity was found to occur during the atrial T-wave.

5. It is suggested that an "excitation time" theory may account for the normal delay in impulse propagation across the atrio-ventricular junction of the turtle heart.

6. Because of the direction of the lines of electrotonic current flow along the surface of a ventricular element which is considered as being completely external and extracellular to an abutting atrial element, ventricular excitation would normally not be expected to occur during the arrival or rise of the atrial action potential at the junction. With such a fiber arrangement ventricular response would occur at or before a moment roughly that at which there is the most rapid recovery of the responsible atrial element, immediately adjacent to the junction.

7. Preparations showing long A-V delays which approach multiples of the normal value have been observed to show responses of small tissue regions which were localized near the A-V junction and which showed onset of activity at times intermediate between those of atrial and of ventricular responses.

8. It is suggested that the short P-R interval of the Wolff-Parkinson-White syndrome may represent atrio-ventricular conduction without the usual, normal junctional pause in the spread of excitation.

## CONCLUSIONS

It is concluded that an "excitation time" theory may be used to explain the delay normally occurring in the transmission of the cardiac impulse across the atrio-ventricular junction in the turtle heart. Ventricular excitation normally does not occur during or because of the arrival and rise of the atrial action potential but during the period of electrical recovery of the atrium. Long atrio-ventricular delays may involve serial activation of several elements thus multiplying the single junctional pause found in the normal heart.

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# THE APPARENT VOLUME OF DISTRIBUTION OF SULFOCYANATE AND OF SULFANILAMIDE IN THE DOG[1]

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A number of substances, including sodium, chloride, bromide, sulfate, sucrose and sulfocyanate (1, 2, 3, 4) appear in the main to be distributed, following injection, through only the extracellular portion of the body fluids. Of these substances sulfocyanate is most frequently used to measure extracellular fluid volume because of the apparent ease of its determination and its slow excretion.

The distribution of sulfocyanate, however, has usually been followed for only a few hours after injection and only after single injections. The degree of variations in the apparent volume of distribution over longer periods and after multiple injections has not been clearly delineated. Study of such variations in dogs was undertaken because repeated measurements of extracellular fluid volume were desired in experiments extending over many days.

Crandall and Anderson (4) determined the sulfocyanate distribution volume in dogs 2 to 4 hours after injection. Lavietes, Bourdillon and Klinghoffer (3) obtained a constant volume in human subjects as long as 24 hours after injection. No study has been found of the volume of distribution of sulfocyanate in the dog over a similar period of time.

In six preliminary determinations of the volume of distribution in four dogs at intervals of 16 to 21 hours after injection, values were obtained ranging from 29 to 40 per cent of the body weight with an average of 36.8 per cent. In four determinations at 4 hours the average volume was 32.7 per cent of the body weight. This latter value is identical with that obtained by Crandall and Anderson as the average in 33 dogs, and is somewhat closer to the values calculated from the total water and electrolyte content of whole dogs (1). These findings suggested that the apparent volume of distribution of sulfocyanate in the dog increased with time.

Painter (5) has reported that the volume of distribution of sulfanilamide in the dog may be used as a measure of the total body water. Heinemann (6), however, found that sulfanilamide is not distributed evenly between serum and cells of human blood. If such is the case in dog blood in vivo, the distribution of sulfanilamide, calculated as Painter does, from its concentration in whole blood, cannot be used as a valid measure of total body water. It seemed worthwhile, therefore, to reinvestigate this problem, taking care to determine separately the concentration of sulfanilamide in cell water and in serum water.

METHODS. Sulfanilamide was determined in serum and in urine by the

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[2] National Research Council Fellow in the Medical Sciences.

method of Bratton and Marshall (7). For the determination of sulfocyanate the method of Crandall and Anderson (4) was modified as follows:

The concentration of sulfocyanate was determined from the depth of color developed with ferric nitrate reagent. In the preliminary experiments referred to above, the reagent was added to a trichloroacetic acid filtrate of serum and to urine from which the pigment had been removed with animal charcoal, and the depth of color read in a visual colorimeter. The animal charcoal was later found to remove some of the sulfocyanate.

Therefore, to obtain more reliable determinations of sulfocyanate for the present experiment the method was adapted to the Evelyn photoelectric colorimeter.

Reagents. 1. Ten per cent trichloroacetic acid. 2. Ferric nitrate reagent consisting of: 25 grams of $Fe(NO_3)_3 \cdot 9H_2O$, 12.5 cc. of $HNO_3$, 500 cc. of water.

*Serum.* A protein-free filtrate of serum was obtained by adding 6 cc. of 10 per cent trichloroacetic acid to 2 cc. of serum. Four cubic centimeters of the filtrate were transferred to a calibrated colorimeter tube, followed by 4 cc. of distilled water and 2 cc. of ferric nitrate reagent, making a total volume of 10 cc. A blank was prepared simultaneously consisting of 5 cc. of distilled water, 3 cc. of 10 per cent trichloroacetic acid, and 2 cc. of ferric nitrate reagent. The readings were made with a filter of 490 $\mu$.

*Urine.* Five-tenths cubic centimeter of urine, 7.5 cc. of distilled water, and 2 cc. of ferric nitrate reagent were pipetted into a colorimeter tube making a total volume of 10 cc. This solution was a 1:20 dilution of the urine. At this dilution the effect of urine pigment was found to be negligible when the undiluted urine was of a specific gravity of 1.010 or less. The blank was made up of 8 cc. of water and 2 cc. of ferric nitrate reagent. The readings were made with the filter of 490 $\mu$.

A blank substance which produced color with the ferric nitrate reagent, but which was not oxidizable under the same conditions as sulfocyanate, was found in the urine of dogs which had received no sulfocyanate. The concentrations of this substance in the urine varied directly with the specific gravity, and interfered with the recovery of added sulfocyanate when the specific gravity of the urine was higher than 1.005. Because of this fact and because of urinary pigment unknown urines were initially diluted 1:5 or 1:10 to a specific gravity of 1.005 or less. This dilution plus the second 1:20 dilution in the colorimeter tube made a final dilution of 1:100 or 1:200.

In 27 urine specimens from 7 normal dogs it was found that for every 0.001 of specific gravity the average color value of this blank substance was equivalent to that produced by 0.146 mgm. per cent of sulfocyanate with a standard deviation of $\pm 8.0$ per cent. From this relationship a value for the blank was calculated for each unknown urine. The accuracy of recovery of known amounts of sodium sulfocyanate added to 37 urine specimens with a dilution of 1:100 or 1:200 was $\pm 5.0$ per cent.

A small blank of a magnitude equivalent to 0.1 to 0.4 mgm. per cent sulfocyanate was found in the serum of dogs which had received no sulfocyanate.

This blank, however, did not appear to be additive to sulfocyanate added to the serum in vitro. If the blank was excluded, the recovery of the sulfocyanate varied from 105 to 96 per cent. If the blank was included the recovery was 93 to 98 per cent. For the purposes of the experiment this blank was considered not to be additive and the accuracy of the determination was taken to be ±5 per cent. The behavior of the blank remains an enigma.

PROCEDURE AND CALCULATION. Sodium sulfocyanate in 1 per cent solution was injected intravenously into the dog from a calibrated burette. At each time for the determination of the volume of distribution a sample of blood was taken from the jugular vein, the urine was collected from the metabolic cage, the animal was catheterized and the bladder washed several times with isotonic saline solution. The feces of dogs 3 and 4 were collected over the entire period of the experiment and analyzed; a negligible amount of color-producing substance was present. Anesthesia was not required.

The calculation of the volume of distribution of diffusible sulfocyanate presents some difficulties. Lavietes, Bourdillon and Klinghoffer (3) found that the concentration of sulfocyanate was approximately 10 per cent higher in serum than in transudates in patients given sulfocyanate. Rosenbaum and Lavietes (8) showed that in human serum a fraction of sulfocyanate is bound to lipoid and is not present in the ultrafiltrate. To allow for this non-diffusible fraction in serum and for the entrance of sulfocyanate into the red cell, the former authors constructed an equation which was felt to represent more nearly the extracellular fluid volume. This equation was not used for the calculation in our experiment because no direct measurements were available of the blood volume and serum volume.

The apparent volume of distribution of any substance theoretically may be calculated from the following equation:

$$\text{Apparent volume of distribution} = \frac{\text{Amount given} - \text{amount excreted}}{\frac{\text{Rise in serum concentration}}{\text{Water content of serum}}} \times \text{Donnan factor} \times (1\text{-Fraction bound})$$

Theoretically, the Donnan factor and (1—fraction bound) are separate entities, the latter depending upon the amount of substance so bound to protein or lipoid that it will not pass through the capillary membrane. As actually measured by ultrafiltration the diffusible fraction is the product of these two factors. For sulfocyanate in dog serum the diffusible fraction is approximately 0.83 x serum water concentration (9). This factor in part cancels the correction for the water content of serum, 1:0.93. For this reason and since no better methods of calculation are available at present, the following simplified equation was used for the calculation in this experiment:

$$\text{Apparent volume of distribution} = \frac{\text{Amount given} - \text{amount excreted}}{\text{Rise in serum concentration}}$$

Sulfanilamide in 0.6 per cent solution was injection and recovered in the same manner as sulfocyanate. As sulfanilamide is excreted much more rapidly than

sulfocyanate, the theoretical volume of distribution at the time of injection was calculated from a concentration value obtained by extrapolation of the con-

TABLE 1

*The apparent volume of distribution of sodium sulfocyanate in two dogs during 96 hours after three successive injections*

| DOG | WEIGHT | TIME | | NaSCN | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | After initial injection | After immed. injection | Amount given i.v. | Serum conc. | Output | | Net retention | Volume of distribution | |
| | | | | | | Blood | Urine | | | |
| | *kgm.* | *days* | *hours* | *mgm.* | *mgm. per cent* | *mgm.* | *mgm.* | *mgm.* | *liters* | *per cent body weight* |
| 1 | 16.24 | 0 | 0 | 466 | 0.15 | | | | | |
| | | | 4 | | 10.85 | | 11 | 455 | *4.19* | *25.8* |
| | 16.10 | 1 | 24 | | 6.80 | 1 | 107 | 347 | 5.10 | 31.7 |
| | 16.18 | 2 | 48 | | 4.07 | 1 | 108 | 238 | 5.85 | 36.2 |
| | 16.12 | 3 | 72 | | 2.62 | 1 | 64 | 173 | 6.60 | 40.9 |
| | 15.82 | 4 | 96 | | 1.75 | | 29 | 144 | 8.23 | 52.0 |
| | | 10 | | | 0.34 | | 51* | 93 | | |
| | 15.66 | 10 | 0 | 466 | | | | | | |
| | | | 4 | | 11.35 | | 1 | 465 | *4.10* | *26.2* |
| | 15.54 | 11 | 24 | | 9.90 | 1 | 58 | 406 | 4.10 | 26.4 |
| | | 12 | 48 | | | 2 | 95 | 309 | | |
| | 15.22 | 13 | 72 | | 7.07 | | 33 | 276 | 3.91 | 25.7 |
| | 15.66 | 14 | 96 | | 5.10 | 1 | 44 | 231 | 4.53 | 29.0 |
| | | 14 | 0 | 280 | | | | | | |
| | | | 4 | | 10.53 | 1 | 51 | 228 | *4.19* | *26.8* |
| 2 | | 0 | 0 | 493 | 0.19 | | | | | |
| | 15.96 | | 4 | | 8.97 | | 19 | 474 | *5.29* | *33.2* |
| | 16.30 | 1 | 24 | | 4.60 | 1 | 168 | 305 | 6.63 | 40.7 |
| | 16.40 | 2 | 48 | | 1.29 | 1 | 117 | 187 | 14.5 | 88 |
| | | 3 | 72 | | | | 10 | 177 | | |
| | 15.76 | 4 | 96 | | 0.29 | | 19 | 158 | 54.5 | |
| | | | 0 | 491 | | | | | | |
| | 15.74 | | 4 | | 7.92 | | 70 | 421 | *5.31* | *33.7* |
| | 16.20 | 5 | 24 | | 3.06 | 1 | 117 | 303 | 9.90 | 61.1 |
| | 16.40 | 6 | 48 | | 1.26 | 1 | 57 | 245 | 19.5 | |
| | | 15 | 0 | 493 | 0.17 | | | | | |
| | 17.00 | | 4 | | 8.05 | | 7 | 486 | *6.03* | *35.3* |
| | 17.10 | 16 | 24 | | 4.95 | 1 | 149 | 336 | 6.79 | 39.7 |
| | | 17 | 48 | | | 1 | 170 | 165 | | |
| | | 18 | 72 | | 0.59 | | 49 | 116 | 19.6 | |

* Sulfocyanate had disappeared from the urine by the 10th day.

centration attained over the first four hours after injection. The apparent volume of distribution was calculated in three ways, from the concentration of

sulfanilamide in the water of serum, in the water of the red cells, and in the water of whole blood respectively. For the last two calculations the following simple equation was used:

$$\text{Apparent volume of distribution} = \frac{\text{Amount retained}}{\text{Concentration in mgm. per liter of water}}$$

However, for the calculation based on the concentration of sulfanilamide in serum water it was necessary to introduce another factor. Heinemann (6) has shown that only 75 per cent of sulfanilamide in human serum is ultrafiltrable

TABLE 2

*The apparent volume of distribution of sodium sulfocyanate in two dogs during 96 hours after a single injection*

| DOG | WEIGHT | TIME AFTER INJECTION | | NaSCN | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Amount given i.v. | Serum conc. | Output | | Net Retention | Volume of distribution | |
| | | | | | | Blood | Urine | | | |
| | *kgm.* | *days* | *hours* | *mgm.* | *mgm. per cent* | *mgm.* | *mgm.* | *mgm.* | *liters* | *per cent body weight* |
| 3 | | 0 | 0 | 260 | 0.10 | | | | | |
| | 10.98 | | 4 | | 6.18 | 1 | 0 | 259 | 4.18 | 38.1 |
| | 10.92 | 1 | 24 | | 4.62 | 1 | 58 | 200 | 4.33 | 39.7 |
| | 10.78 | 2 | 48 | | 2.73 | 1 | 91 | 108 | 3.96 | 36.8 |
| | 10.88 | 3 | 72 | | 1.88 | | 25 | 83 | 4.42 | 40.6 |
| | 10.84 | 4 | 96 | | 1.38 | | 27 | 56 | 4.06 | 37.4 |
| | | 14 | | | | | 95 | −39 | | |
| 4 | | 0 | 0 | 260 | 0.15 | | | | | |
| | 11.28 | | 4 | | 7.18 | 2 | 0 | 258 | 3.59 | 31.8 |
| | 11.38 | 1 | 24 | | 4.95 | 1 | 45 | 212 | 4.28 | 37.6 |
| | 11.76 | 2 | 48 | | 2.50 | 1 | 93 | 118 | 4.72 | 40.1 |
| | 11.46 | 3 | 72 | | 1.73 | | 15 | 103 | 5.95 | 52.0 |
| | 11.40 | 4 | 96 | | 1.36 | | 52 | 51 | 3.75 | 32.9 |
| | | 14 | | | | | 116 | −65 | | |

the rest being bound presumably to protein. Therefore the following equation was used:

$$\text{Apparent volume of distribution} = \frac{\text{Amount retained}}{\text{Concentration in mgm. per liter serum water} \times 0.75}$$

The water content of whole blood was determined directly by the difference between wet and dry weights, that of the red cells was calculated from the water content of whole blood and the cell volume. The water content of serum was calculated from the total protein concentration.

RESULTS. The volume of distribution of sulfocyanate was followed after three successive injections in two dogs over periods of 24 to 96 hours (table 1).

The values obtained at 4 hours after injection were fairly constant in each dog. With the exception of the second injection in dog 1, the volumes at 24 hours were definitely larger than at 4 hours, and increased steadily up to 96 hours. The values obtained after the second and third injection were calculated on the assumption that all of the sulfocyanate excreted was from the imme-

TABLE 3

*The apparent volume of distribution of sodium sulfocyanate in two dogs at one, two, and four hours after three successive injections*

| DOG | WEIGHT | DATE | TIME AFTER INJECTION | NaSCN | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Amount given i.v. | Serum conc. | Output | | Net retention | Volume of distribution | |
| | | | | | | Blood | Urine | | | |
| | *kgm.* | | *hours* | *mgm.* | *mgm. per cent* | *mgm.* | *mgm.* | *mgm.* | *liters* | *per cent body weight* |
| 3 | | May 25 | 0 | 289 | 0.20 | | | | | |
| | 11.04 | | 1 | | 7.68 | | | 289 | 3.76 | 34.0 |
| | 11.01 | | 2 | | 7.62 | 2 | | 287 | 3.77 | 34.2 |
| | 10.94 | | 4 | | 7.38 | 1 | 5 | 281 | 3.80 | 34.7 |
| | | June 2 | 0 | 289 | 0.17 | | | | | |
| | 11.50 | | 1 | | 8.25 | | | 289 | 3.50 | 30.4 |
| | 11.70 | | 2 | | 7.43 | 2 | | 287 | 3.86 | 33.0 |
| | 11.50 | | 4 | | 7.00 | 2 | 17 | 268 | 3.83 | 33.3 |
| | | June 29 | 0 | 290 | 0.30 | | | | | |
| | 10.20 | | 1 | | 8.16 | 1 | | 289 | 3.54 | 34.7 |
| | 10.14 | | 2 | | 8.00 | 2 | | 287 | 3.59 | 35.4 |
| | 10.10 | | 4 | | 7.79 | 3 | 7 | 277 | 3.56 | 35.2 |
| 4 | | May 25 | 0 | 289 | 0.23 | | | | | |
| | 11.38 | | 1 | | 8.06 | | | 289 | 3.58 | 31.5 |
| | 11.34 | | 2 | | 8.32 | 2 | | 287 | 3.45 | 30.4 |
| | 11.28 | | 4 | | 8.16 | 1 | 3 | 283 | 3.47 | 30.8 |
| | | June 2 | 0 | 289 | 0.12 | | | | | |
| | 11.52 | | 1 | | 8.85 | | | 289 | 3.26 | 28.5 |
| | 11.42 | | 2 | | 8.44 | 2 | | 287 | 3.40 | 29.8 |
| | 11.44 | | 4 | | 8.05 | 2 | 17 | 268 | 3.33 | 29.1 |
| | | June 29 | 0 | 289 | 0.34 | | | | | |
| | 10.50 | | 1 | | 9.35 | 1 | | 288 | 3.08 | 29.4 |
| | 10.42 | | 2 | | 9.30 | 3 | | 285 | 3.07 | 29.5 |
| | 10.36 | | 4 | | 8.75 | 4 | 4 | 277 | 3.16 | 30.5 |

diately preceding injection only. If a portion were derived from previous injections, the volumes obtained, and hence the discrepancies, would be even larger.

After the second injection in dog 1 the apparent volume of distribution remained at a constant level for 72 hours before increasing. In this dog 79 per

cent of the injected sulfocyanate was recovered by the end of the eighth day after the last injection, but sulfocyanate was still being excreted.

The apparent volume of distribution of sodium sulfocyanate after a single injection was followed for 96 hours in two other dogs (table 2). In dog 3 the values obtained over four days varied only slightly, and in both directions from the 4 hour volume. In dog 4 a large increase in the apparent volume occurred during the first 72 hours. On the fourth day the excretion rate increased and the apparent volume returned to approximately the 4 hour value.

Urine and stools were collected for 14 days after the administration of sulfocyanate. In both dogs more sulfocyanate was recovered than was given, 115

TABLE 4

*The distribution of sulfanilamide between cells and serum in dog blood in vivo, and the apparent volume of distribution of sulfanilamide calculated from the respective concentrations in whole blood water, in serum water, and in red cell water*

| DOG | TIME | WEIGHT | AMOUNT GIVEN | SULFANILAMIDE | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Concentration | | | Net retention | Volume of distribution | | | | | |
| | | | | Blood water | Serum water | Red cell water | | Blood water | | Serum water | | Red cell water | |
| | *hour* | *kgm.* | *mgm.* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm.* | *liter* | *per cent body weight* | *liter* | *per cent body weight* | *liter* | *per cent body weight* |
| 5 | 0 | 5.57 | 240 | 7.0* | 5.0* | 10.3* | 240 | 3.4 | 62 | 6.3 | 113 | 2.3 | 42 |
| | 1 | | | 6.1 | 4.5 | 8.8 | | | | | | | |
| | 2 | | | 5.1 | 3.9 | 7.2 | | | | | | | |
| | 4 | 5.52 | | 4.4 | 3.0 | 6.8 | 183 | 4.2 | 76 | 8.0 | 145 | 2.7 | 49 |
| | 12 | 5.72 | | 1.4 | 0.7 | 2.6 | 101 | 7.2 | 126 | 20.2 | 354 | 4.0 | 70 |
| 6 | 0 | 7.30 | 600 | 11.3* | 9.1* | 15.1* | 600 | 5.3 | 73 | 8.8 | 121 | 4.0 | 55 |
| | ½ | | | 11.0 | 8.9 | 14.8 | | | | | | | |
| | 1 | | | 9.6 | 7.9 | 13.1 | | | | | | | |
| | 2 | | | 8.7 | 7.1 | 11.7 | | | | | | | |
| | 3 | | | 8.1 | 6.5 | 11.2 | | | | | | | |
| | 4 | 7.30 | | 7.2 | 6.1 | 9.1 | 509 | 7.0 | 96 | 11.1 | 152 | 5.6 | 77 |
| | 12 | 7.20 | | 4.1 | 3.2 | 5.6 | 338 | 8.3 | 115 | 14.1 | 196 | 6.0 | 83 |

* Values obtained by extrapolation.

per cent and 125 per cent in dogs 3 and 4 respectively. Only a negligible amount of sulfocyanate was recovered in the stools.

In the same two dogs the apparent volume of distribution of sulfocyanate was measured at 1, 2, and 4 hours after each of three successive injections (table 3). The volume at 1 and 2 hours was calculated by dividing the amount given by the serum concentration. At 4 hours the calculation was modified by subtracting from the amount of sulfocyanate given the amount excreted in the urine (as obtained by catheterization). The values obtained indicated a fairly uniform volume during the first four hours. In the one exception, the second injection of dog 3, through an error the animal was allowed a drink of water.

The results as related to body weight were also in good agreement for all three injections.

Sulfanilamide was found to be unevenly distributed between serum and cells in the blood of two dogs in vivo (table 4). The concentration in cell water was about twice that in serum water. The apparent volume of distribution at the time of injection, when calculated from the concentration in serum water was 113 per cent and 121 per cent of the body weight, and when calculated from the concentration in red cell water was 42 per cent and 55 per cent of the body weight. When calculated from the concentration in the water of whole blood, the values obtained were 62 per cent and 73 per cent of the body weight. The apparent volume of distribution as calculated by all three methods progressively increased in magnitude at the fourth and twelfth hours after injection. Throughout the experiment no acetylated sulfanilamide was found in blood or in urine.

Discussion. The apparent volume of distribution of sulfocyanate appears to be consistently reproducible in any given dog *during the first four hours* after any number of injections. No evidence is presented as to the exact relation of this volume to the true extracellular fluid volume, and hence the former can only be taken as a measure of the approximate, but not the absolute, magnitude of the latter. But *changes* in the distribution volume of sulfocyanate as determined during the first four hours after injection may reasonably be taken to measure *changes* in the true extracellular fluid volume. For this purpose it should be a valid experimental technique.

The evidence presented, however, indicates that *after* the first four hours following injection the volume of distribution of sulfocyanate is no longer an accurate measure of any one fraction of the body fluids. The increase of the apparent volume of distribution over time in these dogs indicates a discrepancy between the apparent volume and the true volume of distribution because there was no valid reason to believe that the latter was increasing. To explain this discrepancy it is necessary to examine the assumptions on which the calculation of the apparent volume of distribution is based.

The calculation of the volume of distribution of an injected substance depends upon the following assumptions, as stated by Winkler and Smith (10): "1) that the substance is neither formed nor destroyed in the body; 2) that it is uniformly distributed throughout some portion of the body water; 3) that the concentration of the water of serum is a fair sample of its concentration throughout this portion; 4) that it is excreted solely by way of the urine" and feces.

The experimental evidence indicates little if any destruction of sulfocyanate in the dog. The first assumption, therefore, appears valid to the extent that sulfocyanate does not appear to be destroyed. If any is formed in the body it must be in amounts too small to affect the course of the experiment.

The accuracy of the modified method and the complete recovery over time of the injected substance would appear to preclude recovery from the urine and feces as an explanation of the discrepancy. The fourth assumption likewise appears valid.

The fault must, therefore, lie with one or both of the two remaining assumptions. Either sulfocyanate is not uniformly distributed throughout some portions of the body water, or its concentration in the water of serum is not a fair sample of its concentration throughout this portion. The latter possibility might explain an error in calculating the *absolute* magnitude of the extracellular fluid, but it could hardly explain an *increasing* apparent volume of distribution. On the other hand a rapid diffusion of sulfocyanate into the extracellular fluid, followed subsequently by a slow entry of sulfocyanate into some tissue phases not in equilibrium with the extracellular fluid would readily explain this rising apparent volume. Sulfocyanate is known to enter erythrocytes and some cells of the gastrointestinal mucosa (3, 4), so such an explanation is not without experimental support. The constancy of volume for 72 hours following the second injection of sulfocyanate in dog 1 is consistent with a "saturation" of such a cellular depot, while the greatly increased excretion of sulfocyanate during the fourth day of dog 4 might have been due to the release of such a deposit.

Whatever the exact cause of these fluctuations in apparent volume of distribution, the fact of their existence renders questionable the equation of the apparent volume of distribution of sulfocyanate with any one fraction of the body fluids more than a few hours after injection.

The identification of the apparent volume of distribution of sulfanilamide with total body water has no experimental support whatsoever. If it is calculated on the basis of its concentration in either serum water or cell water, the results are absurdly high or low respectively. Painter's use of the concentration of sulfanilamide in whole blood water to calculate its apparent volume of distribution lacks physiological justification, since the indicated concentration is present neither in the serum phase nor in the cell phase. The results are dependent on the fortuitous combination of cell concentration, serum concentration, and cell volume. Figures so obtained may be approximately of the correct magnitude but are necessarily without physiological meaning.

Waterhouse and Shannon (11) have published experiments leading to the same conclusion. Using the concentration in serum water for the calculation they obtained apparent volumes of distribution of sulfanilamide ranging from 88 to 102 per cent of the body weight. However, they did not take into account the corrections for the non-diffusible fractions in serum and hence obtained lower values than were found in our experiment.

## SUMMARY

A modification is described of the method of Crandall and Anderson for the determination of sulfocyanate in serum and urine of the dog.

Comparable values were obtained at four hours after injections of sulfocyanate in two dogs which were given three successive injections.

Uniform values were also obtained at one, two and four hours after three successive injections in two other dogs.

In three of four dogs the apparent volume of distribution of sulfocyanate increased with time over 24 to 96 hours after injection.

The data indicate that in the dog the apparent volume of distribution of sulfocyanate determined between one and four hours after injection measures a definite fraction of the body fluids which approximates the extracellular fluid volume.

The data also indicate that the apparent volume of distribution of sulfocyanate in the dog is not a valid measure of any one fraction of the body fluids after the fourth hour following injection.

Sulfanilamide was found to be unevenly distributed between serum and cells in dog blood in vivo. The distribution of sulfanilamide cannot be used to measure total body water or any fraction thereof.

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# ABNORMAL CAPILLARY RESISTANCE IN SWINE SUFFERING FROM AN INHERITED BLEEDING DISEASE[1]

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During the last few years a strain of swine owned by the Missouri Agricultural Experiment Station has been under observation because of severe bleeding tendencies. Many of the animals bleed to death from trivial wounds or from apparently spontaneous hemorrhage. Several of the characteristics of the abnormality have been described: the disease is transmitted by both sexes as a Mendelian recessive (1); the blood clotting mechanism is defective (2, 3), and the saline bleeding time is abnormally prolonged (4).

The discovery of an abnormal saline bleeding time has prompted us to test the capillary resistance of the bleeder animals, for in man low capillary resistance and abnormal bleeding times often occur together (5).

METHODS. Capillary resistance was measured by the Dalldorf suction method (6). Negative pressures accurate to within $\pm 0.5$ cm. of mercury were obtained with either a vacuum pump, or with a water aspirator (filter pump), connected to a mercury valve (7) and a mercury manometer. Because of frequent breakage of the glass suction cup designed by Dalldorf (6), we substituted a rubber tube with a bore of 1 cm. and a wall thickness of 8 mm. The animals were strapped down securely on their backs, and the suction tube was applied to the inside of the thigh 1 to 3 inches from the midline.

In preliminary experiments 9 purebred Poland China swine 4 to 6 months of age were tested. The 4 bleeder animals gave 5 or more petechiae when a negative pressure of 75 cm. of mercury was applied for 2 minutes. Four of the 5 normal animals, however, gave no petechiae at this maximum negative pressure. It was therefore impossible to measure quantitatively the normal capillary resistance of the dark-skinned Poland Chinas, and we conducted subsequent experiments on white, or lighter-skinned, Chester White-Poland China crosses. These animals were obtained by breeding 2 white-skinned Chester White-Poland China sows (litter mates carrying the bleeder gene (1)) to male 1, a Poland China bleeder described in a previous publication (4). A total of 11 offspring was obtained in the 2 litters. It was found that all of these closely related animals had capillary resistances that were measurable. Seven of the animals were white, and 4 were black. The white animals were devoid of pigment, and the black animals had very light skins on the inside of the thighs, so that it was easy to detect and count the petechiae. The 11 animals were used for capillary resistance tests when the group had reached the age of 4 months and the individuals weighed 72 to 95

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lbs. Male 6 died before the tests were completed. Preliminary tests, however, indicated that his capillary resistance was abnormally low. Female 11 was discarded because she was a borderline type of animal (1), *i.e.*, neither a severe bleeder nor completely normal.

RESULTS. To secure quantitative information on the bleeding time in the animals chosen for study, saline bleeding time tests were run at weekly intervals for 3 weeks, using the technique previously described (4). The data obtained are shown in table 1. The animals F5, M4 and F1 are from one litter, the remaining animals are from the other litter. The animals fall into 2 groups: the carriers[2], with average saline bleeding times of less than 100 seconds, and the bleeders, with average saline bleeding times of more than 300 seconds.

An experiment on the relative incidence of petechiae in the carriers and bleeders was carried out in conjunction with the saline bleeding time tests. Eight of the animals were grouped into pairs of 1 carrier and 1 bleeder. The carrier animals

TABLE 1

*The saline bleeding time in Chester White-Poland China swine*

| WEEK | CARRIERS | | | | | BLEEDERS | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F8 | F10 | F5 | M4 | M7 | F1 | F9 | F7 | F14 |
| | *sec.* | *sec.* | *sec.* | *sec.* | *sec.* | *sec.* | *sec.* | *sec.* | *sec.* |
| 1 | 75 | 120 | 65 | 120 | 75 | 600+ | 270 | 330 | 600+ |
| 2 | 90 | 100 | 65 | 60 | 60 | 270 | 600+ | 295 | 160 |
| 3 | 85 | 63 | 36 | 80 | 40 | 600+ | 390 | 600+ | 180+* |
| 4 | 67 | 95 | 62 | | 60 | 600+ | 600+ | 600+ | |
| Average... | 79 | 94 | 57 | 87 | 59 | 517+ | 465+ | 456+ | 313+ |

* F14 developed a lung hemorrhage 180 seconds after the saline bleeding time test had been started, and died in about 10 minutes. Autopsy showed 2 small tears on the surface of the right lung, and blood in the thoracic cavity.

F8, F10, F5 and M4 were matched respectively with the bleeders F1, F9, F7 and F14. The *highest* negative pressure that produced no petechiae in the carrier animal was determined at weekly intervals. The bleeder was then tested at the same negative pressure and the number of petechiae counted. Negative pressures of 10 to 50 cm. of mercury (at intervals of 10 cm.) were applied to the inside of the thigh for 2 minutes. The data in table 2 show that the bleeders *consistently* developed petechiae at negative pressures that did not produce petechiae in the carriers.

After the above studies had been made, capillary resistance tests based on Elliott's definition were performed on the animals shown in figure 1. According to his definition (8) capillary resistance is the *lowest* negative pressure (expressed

[2] These animals have normal saline bleeding times (4), and do not have hemorrhagic tendencies. Genetically they are not pure "normals", since they carry the recessive bleeder gene. Inasmuch as they do not suffer from the bleeding disease, they are very satisfactory controls in the present studies.

in cm. of mercury) that is capable of producing 2 or more macroscopic petechiae in a skin area 1 cm. in diameter. The negative pressure is applied for 1 minute. In our experiments, the animals were tested daily for 8 consecutive days. On days 1, 3, 5 and 8, the determinations were made in the afternoon, and on the remaining days in the forenoon. The majority of the white animals showed a daily variation in resistance. The capillary resistance was usually 10 to 15 cm. lower in the afternoon than in the morning.

Figure 1 shows that the capillary resistance, as defined by Elliott, ranged from 35 to 60 cm. in the carrier animals, and from 5 to 35 cm. in the bleeders. The

TABLE 2

*Number of petechiae in the bleeder animals at the highest negative pressure that produced no petechiae in the carriers*

| WEEK | PETECHIAE IN: | | | |
|---|---|---|---|---|
| | F1 | F9 | F7 | F14 |
| | *no.* | *no.* | *no.* | *no.* |
| 1 | 25 | 11 | 3 | 15 |
| 2 | 25 | 1 | 6 | 23 |
| 3 | 20 | 3 | 2 | 32 |
| 4 | 18 | 1 | 10 | |

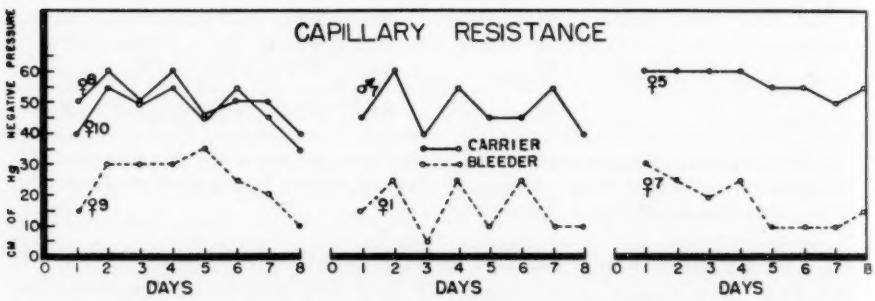


Fig. 1. A comparison of the capillary resistance of carrier and bleeder swine

average capillary resistance for the 8 day period was 51 cm. in the 4 carriers, and 19 cm. in the 3 bleeders, a difference in average resistance of 32 cm.

Discussion. Since similar methods were employed, it is possible to compare our findings (fig. 1) with resistance studies made on man by Elliott (8). He found that the capillary resistance (measured on the forearm) was in the range of 10 to 30 cm. in cases of human idiopathic thrombocytopenic purpura. The bleeder swine (fig. 1) have resistance values that fall in this same range. The carrier swine, however, have a range of capillary resistance, and an average capillary resistance, that are somewhat higher than those found in normal man (8). The differences in capillary resistance which exist between the carrier and

bleeder swine are therefore as great, or even greater, than those found by Elliott to exist between normal and purpuric humans.

SUMMARY

Evidence is presented to show that the capillary resistance is abnormally low in swine suffering from an inherited bleeding disease.

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# METABOLISM OF ASPHYXIATED SPINAL CORD

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It has been found in this laboratory that when the spinal cord of a cat is asphyxiated for 55 to 65 minutes, there is a temporary return of some reflex activity (extensor tone and tendon reflexes) beginning 1½ to 4 hours after the end of asphyxiation, and lasting up to 48 hours (van Harreveld and Marmont, 1939). After this temporary return, reflex activity usually is absent for the rest of the animal's life. It seemed of interest to follow the metabolic changes of the cord which accompany the above changes. For this reason the oxygen uptake of excised spinal cord has been determined at various intervals after a 60 minute asphyxiation.

After asphyxiation of the cord for 35 minutes some reflexes return temporarily (flexion reflex), but others, such as extensor tone and tendon reflexes return permanently. It has been found (van Harreveld, 1941) that the reflex activity present 14 days after such an asphyxiation is much more resistant to oxygen lack than the reflexes of a normal animal. This increased resistance is not present 48 hours after asphyxiation, but develops slowly in the ensuing days. It was of interest, therefore, to determine also the cord metabolism 2 and 14 days after a 35 minute asphyxiation, when the resistance of reflex activity to oxygen lack is so different.

METHOD. The caudal part of the spinal cord of cats was asphyxiated by a method previously described (van Harreveld and Marmont, 1939). The caudal part of the dural cavity and of the spinal cord was isolated by ligating the dura at the lower thoracic level. The next day the blood circulation in that part of the cord was interrupted by forcing Ringer's solution into the isolated part of the dural cavity at a pressure higher than the blood pressure.

The oxygen uptake of the cord was determined in Warburg respirometers using Ringer-glucose medium, buffered with phosphate to pH 7.4. The caudal part of the cord was removed and divided into four parts; the 6th lumbar, 7th lumbar, 1st sacral and the remaining caudal part consisting of the 2nd and 3rd sacral and the coccygeal segments (referred to as the tail). Each of these four parts was sliced parallel to the long axis of the cord with an apparatus consisting of 10 razor blades spaced at 1.5 mm. The tissue was suspended in individual, tared vessels, and weighed before placing in a bath of 38°C. The first reading was taken 50 minutes after the cord was removed. This allowed 15 to 20 minutes for the vessels to come to temperature equilibrium. The metabolism was calculated from the oxygen uptake between 50 and 80 minutes after removal of the cord, and the results were expressed in cubic millimeters of

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oxygen consumed/100 mgm. of wet tissue/hour. The average oxygen uptake of the spinal cord caudal of L5 was calculated from the weights and oxygen consumption of the 4 parts.

In one series of experiments the parts were minced instead of sliced. To study the metabolism of white matter, a number of experiments were carried out on the optic nerve, which may be considered as central white matter.

TABLE 1

*Metabolism of normal spinal cord in cubic millimeters of $O_2$/100 mgm. tissue/hour*

The value in the last column of tables 1, 4, 5 and 6 is the $O_2$ uptake of the entire region of the cord caudal of L5, and is calculated from the individual weights and oxygen consumption of L6, L7, S1 and the tail.

| NUMBER | L6 | L7 | S1 | TAIL | L6-TAIL |
|---|---|---|---|---|---|
| A. Minced tissue, mature cats | | | | | |
| 1 | 48 | 58 | 80 | 69 | 62 |
| 2 | 49 | 52 | 62 | 58 | 54 |
| 3 | 43 | 54 | 56 | 56 | 51 |
| 4 | 49 | 58 | 55 | 69 | 57 |
| 5 | 49 | 52 | 58 | 66 | 55 |
| Average..... | 48 | 55 | 62 | 64 | 56 |
| B. Sliced tissue, mature cats | | | | | |
| 6 | 54 | 65 | 77 | 96 | 70 |
| 7 | 51 | 66 | 69 | 90 | 67 |
| 8 | 55 | 55 | 83 | 101 | 73 |
| 9 | 56 | 61 | 82 | 89 | 69 |
| 10 | 44 | 54 | 61 | 95 | 69 |
| Average..... | 52 | 60 | 74 | 94 | 70 |
| C. Sliced tissue, young cats | | | | | |
| 11 | 59 | 69 | 72 | 85 | 71 |
| 12 | 67 | 87 | 86 | 95 | 81 |
| 13 | 62 | 76 | 87 | 97 | 79 |
| 14 | 69 | 80 | 86 | 117 | 86 |
| Average..... | 64 | 78 | 83 | 99 | 79 |

RESULTS. A. *Normal spinal cord and optic nerve.* Three points are to be noted which influence the oxygen uptake of normal spinal cord and optic nerve.

1. *Metabolism of sliced and minced tissue.* Table 1, A and B, shows that the oxygen uptake is considerably lower in minced than in sliced spinal cord. This difference is more marked in the optic nerve (table 2). Mincing the nerve reduces its metabolism to a fraction of that of an optic nerve sliced in two, lengthwise. The optic nerve is small enough to be used without slicing; the oxygen uptake of the unsliced nerve was found to be only a little higher than that found

in sliced preparations, showing that slicing has only a small influence on tissue respiration. The values found for minced nerves are of the same order as those of Michail and Benetato (1936) who used human optic nerves. The much greater influence of mincing on the oxygen uptake of the optic nerve than on that of the spinal cord may be due to the firmer consistency of this nerve. Hence more force, causing more damage, is needed to mince the optic nerve.

Since slicing obviously causes less damage than mincing, all studies on the spinal cord were made with sliced preparations. However, since the unsliced optic nerve has the highest metabolism (table 2), this nerve was used without further preparation.

It is of interest to note that the metabolism of unsliced peripheral nerve (median nerve) averaged 15 mm.$^3$ of $O^2$/100 mgm. tissue/hour, as compared to 45 mm.$^3$ for unsliced optic nerve.

TABLE 2

*Metabolism of optic nerves in cubic millimeters of $O_2$/100 mgm. tissue/hour*

| NUMBER | INTACT | SLICED IN TWO | MINCED |
|---|---|---|---|
| 1 | 45 | 38 | |
| 2 | 48 | 45 | |
| 3 | 46 | 41 | |
| 4 | 43 | 41 | |
| 5 | 53 | | 8 |
| 6 | 46 | | 7 |
| 7 | 35 | | 7 |
| 8 | 41 | | 16 |
| 9 | | 42 | 5 |
| 10 | | 35 | 8 |
| Average..... | 45 | 40 | 9 |

2. *Influence of age of the animal.* Table 1, C, shows that the spinal cord of young cats has a higher oxygen uptake than that of adults. The same has been found for the brains of young and adult dogs (Himwich and Fazekas, 1941) and rats (Tyler and van Harreveld, 1942). Only mature animals have been used for the asphyxiation experiments.

3. *Gradient of metabolism.* There is a very marked gradient of respiratory activity in the caudal part of the spinal cord. However, unlike the gradient found in the brain, the more cranial parts of the cord have the lowest activity, while the most caudal part has the highest activity. Tables 1 and 4 show that the oxygen uptake of the "tail" is more than 1½ times as great as that of the 6th lumbar segment. It is therefore of the utmost importance to define, as exactly as possible, the segments on which the metabolism is determined.

B. *The effect of severing optic nerve and spinal cord.* Transection of the optic nerve directly behind the eye results in a drop of its metabolism. The respiration of the optic nerve 3, 7 and 14 days after transection was around 35 mm.$^3$/100 mgm. tissue/hour, whereas the average $O^2$ consumption of the heterolateral nerve, which was left intact, was 46 mm.$^3$ (table 3).

Severing of the spinal cord also results in a drop of its metabolism, which, however, is slight. An oxygen uptake of 70 mm.$^3$ was found for the unsevered cord (table 1, B); the values 1, 3, 7 and 14 days after ligating dura and cord were about 65 mm.$^3$ (table 4). Since severing of the spinal cord is a preliminary step for cord asphyxiation, the latter values are to be considered as controls for the asphyxiated cord.

C. *The effect of asphyxiation of the cord.* 1. *Metabolism of the spinal cord after 60 minutes' asphyxiation.* In these experiments the spinal cord was removed at intervals varying from 0 hour to 14 days after 60 minutes' asphyxiation. In the 0 hour experiments, the cord circulation was not re-established, but it should be pointed out that it takes 50 minutes to prepare and equilibrate the tissues and during most of that period the sliced cord is kept in an oxygen saturated medium. Table 5 shows that the oxygen uptake of the cord, taken

TABLE 3

*Metabolism of normal and degenerated optic nerves in cubic millimeters of $O_2$/100 mgm. tissue/hour*

| NUMBER | NORMAL SIDE | DEGENERATED SIDE | | |
|---|---|---|---|---|
| | | After 3 days | After 1 week | After 2 weeks |
| 1 | 40 | 35 | | |
| 2 | 51 | 35 | | |
| 3 | 47 | 31 | | |
| 4 | 52 | | 33 | |
| 5 | 34 | | 33 | |
| 6 | 55 | | 49 | |
| 7 | 48 | | | 33 |
| 8 | 42 | | | 27 |
| 9 | 48 | | | 34 |
| 10 | 41 | | | 34 |
| Average..... | 46 | 34 | 38 | 32 |

out immediately at the end of asphyxiation, is 60 mm.$^3$ or almost as high as that of the normal isolated cord. The metabolism of the cords removed after intervals of 3, 6 and 12 hours shows a progressing decline. The oxygen uptake of the cord after these various intervals was respectively 56, 48 and 38 mm.$^3$ After an interval of 48 hours, the low level of about 30 mm.$^3$ is reached which is maintained during the next 14 days. This level is about half of that of the normal isolated cord. It can be concluded that the full damaging effect of 60 minutes' asphyxiation on oxygen uptake is not apparent immediately, but develops in the ensuing 12 to 48 hours.

2. *The metabolism of the spinal cord after 35 minutes' asphyxiation.* The metabolism 2 days after 35 minutes' asphyxiation showed an average of 41 mm$^3$.; after 14 days the oxygen uptake had decreased to an average of 31 mm$^3$ (table 6). The oxygen consumption found 14 days after 35 minutes' asphyxiation was not significantly different from that found 2 weeks after 60 minutes' asphyxiation,

though the 35 minute animals, contrary to the 60 minute ones, showed some reflex activity (usually extensor tone).

*Anatomical changes in the cord.* The 5th lumbar segment, adjacent to the part of the cord that had been used for metabolism determination, was fixed in 95 per cent alcohol, sectioned and stained with toluidine blue. These preparations showed changes similar to those described, for instance, by Tureen (1934) in asphyxiated spinal cord. The cord removed immediately after a 60 minute asphyxiation showed a normal appearance. After 3 hours considerable chromatolysis was present, some cells stained poorly, and in many cells the

TABLE 4

*Metabolism of severed spinal cord in cubic millimeters of $O_2$/100 mgm. tissue/hour*

| NUMBER | INTERVAL AFTER TRANSECTION | L6 | L7 | S1 | TAIL | L6-TAIL |
|---|---|---|---|---|---|---|
| 1 | 1 day | 49 | 70 | 66 | 82 | 68 |
| 2 | | 47 | 55 | 64 | 81 | 60 |
| 3 | | 59 | 65 | 73 | 76 | 66 |
| Average | | 52 | 63 | 68 | 80 | 65 |
| 4 | 3 days | 53 | 55 | 60 | 79 | 61 |
| 5 | | 60 | 77 | 72 | 87 | 70 |
| 6 | | 50 | 52 | 70 | 84 | 62 |
| Average | | 54 | 61 | 67 | 83 | 64 |
| 7 | 1 week | 46 | 59 | 62 | 75 | 60 |
| 8 | | 44 | 53 | 56 | 84 | 60 |
| 9 | | 54 | 64 | 70 | 84 | 67 |
| Average | | 48 | 59 | 63 | 81 | 62 |
| 10 | 2 weeks | 52 | 56 | 62 | 81 | 62 |
| 11 | | 46 | 62 | 62 | 95 | 67 |
| 12 | | 65 | 67 | 76 | 85 | 72 |
| Average | | 54 | 62 | 67 | 87 | 67 |

nuclear boundary was not distinct. After 6, and especially after 12 hours, a marked decrease of the number of ganglion cells was apparent. Chromatolysis was about complete and most cells were poorly stained. Infrequent neurophagia was observed. After 48 hours no ganglion cells could be found. There was a slight increase in the interstitital cells, and usually a proliferation of the capillaries. After 7 and 14 days, a considerable increase of the interstitial cells was observed. Most of these cells had a pale, vesicular nucleus; some polymorphonuclear cells were present.

Preparations of the cords removed 2 days after 35 minutes' asphyxiation showed a greatly reduced number of ganglion cells with advanced chromatol-

TABLE 5

*Metabolism of spinal cord asphyxiated for 60 minutes in cubic millimeters of $O_2$/100 mgm. tissue/hour*

| NUMBER | INTERVAL AFTER ASPHYXIATION | L6 | L7 | S1 | TAIL | L6-TAIL |
|---|---|---|---|---|---|---|
| 1 | 0 hour | 47 | 56 | 68 | 85 | 63 |
| 2 | | 50 | 63 | 66 | 71 | 61 |
| 3 | | 48 | 59 | 62 | 72 | 59 |
| 4 | | 48 | 63 | 58 | 70 | 57 |
| Average | | 48 | 60 | 64 | 75 | 60 |
| 5 | 3 hours | 53 | 61 | 57 | 79 | 62 |
| 6 | | 33 | 42 | 43 | 75 | 47 |
| 7 | | 42 | 57 | 65 | 94 | 62 |
| 8 | | 41 | 49 | 61 | 61 | 51 |
| Average | | 42 | 52 | 57 | 77 | 56 |
| 9 | 6 hours | 40 | 37 | 31 | 44 | 39 |
| 10 | | 33 | 47 | 55 | 79 | 55 |
| 11 | | 38 | 49 | 63 | 77 | 55 |
| 12 | | 33 | 42 | 45 | 56 | 44 |
| Average | | 36 | 44 | 49 | 64 | 48 |
| 13 | 12 hours | 40 | 41 | 45 | 53 | 45 |
| 14 | | 23 | 26 | 38 | 47 | 32 |
| 15 | | 27 | 33 | 35 | 47 | 35 |
| 16 | | 38 | 33 | 41 | 51 | 39 |
| Average | | 32 | 33 | 40 | 50 | 38 |
| 17 | 2 days | 31 | 28 | 38 | 46 | 36 |
| 18 | | 23 | 24 | 37 | 37 | 29 |
| 19 | | 27 | 26 | 39 | 33 | 31 |
| 20 | | 23 | 22 | 21 | 31 | 24 |
| Average | | 26 | 25 | 34 | 37 | 30 |
| 21 | 1 week | 29 | 29 | 46 | 52 | 37 |
| 22 | | 34 | 35 | 34 | 32 | 34 |
| 23 | | 23 | 24 | 28 | 35 | 27 |
| 24 | | 25 | 28 | 38 | 35 | 30 |
| Average | | 28 | 29 | 37 | 39 | 32 |
| 25 | 2 weeks | 23 | 18 | 20 | 25 | 22 |
| 26 | | 23 | 18 | 20 | 52 | 30 |
| 27 | | 36 | 30 | 25 | 43 | 31 |
| 28 | | 22 | 25 | 37 | 43 | 31 |
| Average | | 26 | 23 | 26 | 41 | 29 |

ysis. Furthermore, there was increase of the interstitial cells and proliferation of the capillaries. Two weeks after 35 minutes' asphyxiation, the ganglion cells showed regeneration of Nissl substance; about the same number of nerve cells, as found at 48 hours, was present. The same increase of interstitial cells, as found 2 weeks after 60 minutes' asphyxiation, was observed.

A slice of the 5th lumbar segment of the cats surviving 35 or 60 minutes' asphyxiation for 2 weeks, was stained with the Marchi method. Heavy degeneration was found in the anterior and lateral columns; the degeneration in the posterior columns, however, was only slight. It was found previously (van Harreveld, 1940) that 65 minutes' asphyxiation of the cord leaves the

TABLE 6

*Metabolism of spinal cord asphyxiated for 35 minutes in cubic millimeters of $O_2$/100 mgm. tissue/hour*

| NUMBER | INTERVAL AFTER ASPHYXIATION | L6 | L7 | S1 | TAIL | L6-TAIL |
|---|---|---|---|---|---|---|
| 1 | 2 days | 20 | 21 | 26 | 52 | 30 |
| 2 | | 32 | 32 | 39 | 82 | 46 |
| 3 | | 28 | 34 | 42 | 52 | 37 |
| 4 | | 28 | 33 | 41 | 63 | 37 |
| 5 | | 34 | 35 | 44 | 72 | 45 |
| 6 | | 44 | 47 | 61 | 64 | 53 |
| Average | | 31 | 34 | 42 | 64 | 41 |
| 7 | 2 weeks | 19 | 23 | 33 | 60 | 34 |
| 8 | | 34 | 35 | 45 | 54 | 41 |
| 9 | | 17 | 24 | 27 | 38 | 25 |
| 10 | | 25 | 34 | 39 | 39 | 33 |
| 11 | | 17 | 21 | 27 | 41 | 26 |
| 12 | | 20 | 20 | 25 | 41 | 25 |
| Average | | 22 | 26 | 33 | 46 | 31 |

posterior roots intact, which explains the slight degeneration in the pos terior columns.

Discussion. The gradient of metabolic activity found in the normal spinal cord is of interest particularly since it is of the reverse order than that in the brain. This gradient is probably due to the relative amounts of gray and white matter present in the different segments of the cord. The gray matter has a higher respiration rate than the white matter (Holmes, 1930), and it has been shown by Donaldson and Davis (1903) that the relative amount of gray matter in the caudal part of the human spinal cord increases considerably from the cranial to the caudal segments. This increase seems to be great enough to account for the gradient in metabolism.

The Wallerian degeneration of the nerve fibers with disintegration of the myelin sheaths probably causes the drop in metabolism after transection of the

optic nerve. The slight drop in metabolism after transection of the cord may be caused by the degeneration of the descending tracts. The destruction of ganglion cells and the secondary degeneration of their fibers after asphyxiation of the cord will obviously cause a much more marked drop in the oxgen uptake.[2] Although the interstitial cells probably have a low metabolism, their marked increase 1 to 2 weeks after asphyxiation may offset, to a certain extent, the drop due to destruction of ganglion cells and nerve figers.

It was found that the oxygen uptake of the cord returns to almost normal values shortly after a 60 minute asphyxiation, but decreases in the ensuing 12 hours to a low level. This may indicate that for a short period after 60 minutes' asphyxiation, the concentration of the respiratory enzymes is approximately normal. However, since the enzymes cannot be replaced by the structures damaged by asphyxiation, respiration and other functional activities due to these enzymes soon become lost. Parallel with these metabolic changes, reflex activity returns after such periods of asphyxiation, to disappear again a number of hours later (van Harreveld and Marmont, 1939).

The possibility has been considered (van Harreveld, 1941) that the high resistance of reflex activity to oxygen lack shown by cats 14 days after a 35 minute asphyxiation might be due to a very low cord metabolism in these animals, enabling the tissue to function for an unusually long period on the small amount of oxygen present in blood and tissue. At that time it had been argued that this explanation is unlikely, and the present experiments substantiate this view. The difference between the oxygen uptake 48 hours after a 35 minute asphyxiation (when the high resistance to oxygen lack has not yet developed), and after 14 days, is not large, whereas the survival time of the reflex activity at 14 days is many times greater than that at 48 hours.

## SUMMARY

The oxygen consumption of the caudal part of the normal and asphyxiated spinal cord and of the optic nerve was determined by means of Warburg respirometers. It was found that:

1. A gradient of oxygen consumption was present in the region of the cord studied (caudal of L5). The most caudal part had the highest metabolism. This gradient is the reverse of that found in the brain.

2. Wallerian degeneration of the optic nerve and of descending tracts in the spinal cord results in only a moderate decrease of the oxygen uptake.

3. Destruction of the perikarya, with secondary degeneration of the rest of the neurons, by 60 minutes' asphyxiation, causes a marked decrease in oxygen uptake.

4. When the cord is examined immediately after a 60 minute asphyxiation, the metabolism is only slightly lower than in the normal cord. In the ensuing 12 to 48 hours the oxygen uptake drops to about half the value found in the

[2] An increased water content of the optic nerve and cord due to transection (circulatory disturbances) or asphyxiation (capillary damage) may, during the first days, cause additional depression of the metabolism.

unasphyxiated cord. During the rest of the period of observation (two weeks) the metabolism remains at this level.

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# PHOSPHORUS ABSORPTION IN THE ANESTHETIZED DOG AS DETERMINED WITH THE RADIOACTIVE ISOTOPE[1]

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Few measurements are recorded of radioactive phosphorus in the blood and liver soon after the administration of the isotope, i.e., during the period of maximum absorption. Furthermore, since rats and mice have been used almost exclusively for this type of investigation, the reported changes in the P* content of tissues with time are based on values obtained from animals which were sacrificed at the time of sampling. The use of the dog as the experimental animal in this study had the following advantages: 1, samples of liver and of arterial, portal and jugular blood were taken simultaneously; 2, the changes in the isotope content of these tissues were observed on the same animal at different intervals of time.

METHODS. The P* was given as sodium phosphate dissolved in 100 cc. of 0.9 per cent NaCl at pH 6 to 7. Doses, ranging from 542,000 to 4,250,000 counts per minute (scale-of-four Geiger-Müller counters), were introduced through a glass cannula into the duodenum of anesthetized[2] dogs (11 to 23 kgm.). No phosphorus determinations were made since the resultant sacrifice of P* would have been considerable, but the dose probably never exceeded 15 mgm. of phosphorus.

Samples of blood from the portal, jugular and carotid vessels, of liver, and of intestinal contents were taken at intervals up to 4 hours each, and the material weighed as soon as possible. V-shaped pieces were cut from the edge of the liver and the wounds thus made closed immediately with gut sutures. Intestinal contents were removed through the intestinal wall with a hypodermic needle. When a urine sample was desired, the bladder was washed out with saline and the washings added to the urine which had been excreted during the period. At the end of the experiment the small intestine was removed and washed out thrice with saline and an aliquot of the washings taken for the P* determination. The liver was removed and weighed to permit a calculation of the total P* present in the organ at any time. One dog had a Thiry-Vella fistula which was washed out with saline at hourly intervals, and the activity of the washings determined. Samples of muscle (biceps femoris) were taken at various intervals.

The samples were dissolved in fuming nitric acid, and the radioactivity of the acid solution measured by the technique of Bale, Haven and LeFevre (1). Results are expressed as the percentages of the original dose per gram of blood, liver, skeletal muscle, and intestinal contents; the values for the urine, and the intestinal and fistula washings, are in terms of the total isotope present.

[1] Supported in part by a grant from the Rockefeller Foundation.

[2] Dial with urethane kindly supplied by the Ciba Pharmaceutical Company.

RESULTS. The mean percentages of the dose $\times 10^{-3}$ per gram of tissue and the specific activities of the tissues are given in table 1. Since determinations of arterial blood and intestinal tissue were made in only two of the eight dogs studied, the values obtained on these two dogs are given separately in table 2. The values for total phosphorus content of blood and liver were taken from Greenwald (2) and Flock, Bollmann and Mann (3), respectively.

*Blood.* A significant amount of P*[3] appeared in the arterial, portal and jugular blood 5 minutes after administration of the isotope and, as table 1 shows, the

TABLE 1

*P* in the blood and liver during absorption from the intestine*

| | TIME (IN MINUTES) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 45 | 60 | 90 | 120 | 150 | 180 | 210 | 240 |
| | Number of experiments | | | | | | | | | | |
| | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| **Portal blood** | | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | 1.89 | 4.24 | 4.94 | 5.22 | 5.63 | 5.00 | 5.65 | 5.68 | 5.97 | 5.45 | 5.70 |
| $10^{-3}$ per cent dose/mgm.P* | 4.61 | 10.34 | 12.05 | 12.73 | 13.73 | 12.20 | 13.78 | 13.85 | 14.56 | 13.29 | 13.90 |
| | Number of experiments | | | | | | | | | | |
| | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| **Jugular blood** | | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | 0.71 | 1.89 | 2.77 | 3.38 | 3.86 | 4.16 | 4.46 | 4.58 | 5.28 | 4.80 | 5.48 |
| $10^{-3}$ per cent dose/mgm.P* | 1.73 | 4.61 | 6.76 | 8.24 | 9.41 | 10.15 | 10.88 | 11.17 | 12.88 | 11.71 | 13.37 |
| | Number of experiments | | | | | | | | | | |
| | | 5 | | 5 | | 5 | | 5 | | 4 | 3 |
| **Liver** | | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | | 3.58 | | 9.41 | | 19.55 | | 28.67 | | 33.83 | 33.40 |
| $10^{-3}$ per cent dose/mgm.P† | | 1.37 | | 3.60 | | 7.46 | | 10.94 | | 12.91 | 12.75 |

* Total P in blood = 0.41 mgm./gram.

† Total P in liver = 2.62 mgm./gram.

greatest change occurred within a half-hour, after which there was only a gradual increase for the duration of the experiment (4 hrs.). The average jugular blood content of P* per milligram of total phosphorus was always less than that of the portal, but this difference became less progressively and was not significant after $3\frac{1}{2}$ hours. As table 2 shows, there is no significant difference in the P* content of the jugular and carotid blood at any of the times studied.

*Liver.* At the earliest time of measurement, 15 minutes after P* administra-

[3] In most of these experiments a count of 3 times background, which is considered significant, represents approximately $1 \times 10^{-5}$ per cent of the dose.

tion, the liver contained a considerable amount of the isotope ($3.6 \times 10^{-2}$ per cent of the dose/gram). The P* content increased rather rapidly for about $2\frac{1}{2}$ hours after which it maintained a fair constancy. The liver greatly exceeds the blood in the amount of P* which it acquires per gram of tissue (table 1). However, per milligram of phosphorus, the liver contains less isotope than the jugular blood for the first hour, and approximates the value for the portal blood after $2\frac{1}{2}$ hours. A comparable relationship has been shown for bone and blood by Manly, Hodge and Manly (4).

TABLE 2

*P* in the blood and liver during absorption from the intestine*

(2 experiments)

| | TIME (IN MINUTES) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 15 | 30 | 45 | 60 | 90 | 120 | 150 | 180 | 210 | 240 |
| **Portal blood** | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | 2.25 | 2.96 | 3.62 | 6.03 | 5.00 | 5.38 | 4.19 | 5.49 | 4.81 | 4.52 |
| $10^{-3}$ per cent dose/mgm.P | 5.49 | 7.22 | 8.83 | 14.71 | 12.20 | 13.12 | 10.02 | 13.39 | 11.73 | 11.02 |
| **Jugular blood** | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | 0.88 | 1.59 | 2.76 | 3.67 | 3.85 | 4.11 | 4.30 | 4.49 | 4.39 | 4.53 |
| $10^{-3}$ per cent dose/mgm.P | 2.15 | 3.88 | 6.73 | 8.95 | 9.39 | 10.02 | 10.49 | 10.95 | 10.71 | 11.05 |
| **Arterial blood** | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | 1.37 | 1.89 | 3.05 | 3.66 | 4.12 | 3.94 | 4.10 | 4.39 | 4.58 | 4.52 |
| $10^{-3}$ per cent dose/mgm.P | 3.34 | 4.61 | 7.44 | 8.93 | 10.05 | 9.61 | 10.00 | 10.71 | 11.17 | 11.02 |
| **Liver** | | | | | | | | | | |
| $10^{-3}$ per cent dose/gram | 3.07 | | 8.06 | | 10.61 | | 36.39 | | 40.51 | 35.65 |
| $10^{-3}$ per cent dose/mgm.P | 1.17 | | 3.08 | | 4.05 | | 13.89 | | 15.46 | 13.99 |

A calculation based on the average percentage of the dose per gram and the average liver weights of four dogs showed that at $3\frac{1}{2}$ hours the liver contained about 15 per cent of the dose.

*Intestinal contents.* Since all but the final volumes were unknown, the P* determinations on intestinal contents gave only the concentration of the isotope. There were some fluctuations in concentration which appear to be fairly well correlated with the portal-jugular difference in concentration of P* (fig. 1). The secondary rise in the portal-jugular difference, which occurs between the

second and third hour, corresponds with the secondary rise in the concentration of the isotope in the intestinal contents at about the same time. Russell and Nasset (5), who fed a test meal to dogs bearing jejunal fistuli, observed a similar secondary rise in the volume of the fistula contents occurring between the second and third hour after feeding.

Cohn and Greenberg (6) showed that P* injected intraperitoneally appeared in the feces and small intestine of the rat. In the present study, the transfer of P* from the bloodstream to the lumen of the gut was demonstrated in a dog

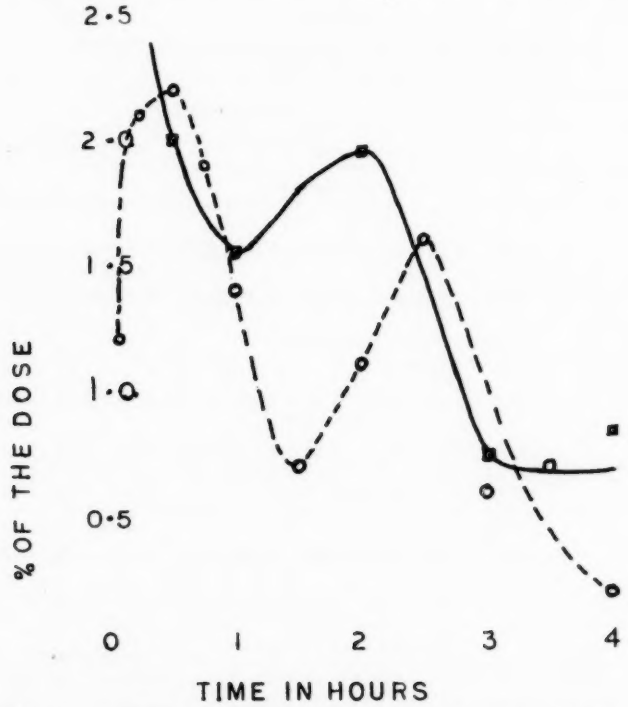


Fig. 1. ○ Average portal-jugular difference × $10^{-3}$ ■ per cent original dose × 10/gram intestinal contents.

bearing a Thiry-Vella fistula of the jejunum. The fistula had been established about 3 months before the animal was used for the P* absorption experiment and there was no trace of blood in the contents obtained from the fistula. It is concluded, therefore, that the P* found was secreted into the lumen as part of the normal succus entericus. The amounts of P* recovered from the Thiry-Vella fistula in four 60-minute periods were: 4.5, 2.3, 1.8, and 0.9 × $10^{-3}$ per cent of the dose, respectively. This indication of a steady decline in secretion of succus entericus is consistent with what is known regarding the response of the small intestine to mechanical stimulation (Nasset, Pierce and Murlin, 7).

From 0.4 to 5 per cent of the P* dose was washed out of the intestine at the end of the experiment, the greater part of which, doubtless, was unabsorbed P*, but certainly some was secreted into the intestine. In one experiment in which the pancreatic and biliary ducts were cannulated, P* was found in the pancreatic juice and the bile 45 minutes after administration of the isotope.

*Urine.* Since the variation was large, little can be said about our observations of the urinary excretion of the isotope: e.g., for the fourth hour, the values ranged from $3 \times 10^{-3}$ to 3 per cent of the dose. The data are very scanty, but there appeared to be a correlation between the volume of urine and the percentage of the dose excreted.

In two experiments in which the urine was collected every fifteen minutes for the first hour, P* first appeared at 45 minutes in one and at one hour in the other.

*Muscle.* The biceps femoris muscle at 4 hours contained P* in amounts ranging from 0.7 to $3.5 \times 10^{-3}$ per cent of the dose per gram of tissue.

DISCUSSION. Under our experimental conditions the absorption of inorganic phosphate appeared to be very rapid; in all instances, 5 minutes after administration there was significant amount of P* in the general circulation. As expected, the portal always contained more P* than the jugular blood. The liver, which averaged 2.4 per cent of the body weight, removed about 1 per cent of the dose from the circulation in 15 minutes and over 15 per cent in $3\frac{1}{2}$ hours. At the end of 4 hours, only 5 per cent of the dose could be washed out of the gut; so 95 per cent had disappeared from the intestinal lumen. At this time the liver contained 15 per cent, the blood 9 per cent, and, if we assume that the P* content of the biceps femoris is representative, the skeletal muscle about 13 per cent. According to the data of Volker and Sognnaes (8) who measured the radioactivity of the bone of these dogs, the skeleton accounted for about 10 per cent and the total dentition about 0.12 per cent. The sum of these figures plus the maximum excretion in the urine (14 per cent) adds up to about $\frac{2}{3}$ of the dose. The other viscera which were not studied could be expected to contain only a small part of the missing 30 per cent of the dose which had disappeared from the gut lumen; so we suspected that a considerable amount of the marked phosphorus was held in the intestinal tissue—probably the mucosa. Other calculations based upon the portal blood flow and the dilution in the intestine required for the observed removal of P* from the gut lumen pointed in the same direction.

According to Grab, Janssen and Rein (9), the average portal blood flow in anesthetized dogs is about 0.5 cc. per gram of liver per minute. In the first thirty minutes of our experiments, therefore, the portal flow was 5 to 7 liters (table 3). If we assume that the portal blood discharged all of its P* before returning, the removal of P* from the lumen of the intestine as indicated by the analysis of the contents would require a portal flow of 15 to 18 liters in 30 minutes. The arterial blood supplying the intestine is not free of P*, and hence the contribution from the intestine is something less than the amount indicated by the P* content of the portal blood.

If the portal-jugular difference be taken as an index of the absorption from the

gut, the portal blood flow necessary to remove the required amount of P* from the lumen is increased 4 to 10 fold. Dilution in the intestine plus absorption could account for some of the change in concentration of P* in the lumen. As introduced the concentration was 1.0 per cent/gram. In thirty minutes, it was reduced to between 0.05 and 0.15 per cent/gram. If an average portal blood flow is assumed and the portal value for P* is taken as a measure of absorption, the dilution required in the intestine is 4 to 14 times the original. The intestine was never extended with fluid as one might expect even with only 400 ml. in the lumen. Using the portal-jugular difference as a measure of P* absorption, of course, increases the dilution required. These discrepancies between the portal blood flow which could be reasonably assumed, and that which could be calculated on the basis of the P* content of the blood and intestinal contents

TABLE 3

*Calculations based on analyses made 30 minutes after P* administration*

| | DOG NUMBER | | |
|---|---|---|---|
| | 3 | 4 | 5 |
| Weight (kgm.) | 19.1 | 17.3 | 13.7 |
| P* × $10^{-3}$ portal blood | 4.80 | 6.29 | 6.47 |
| Portal-jugular difference | 1.62 | 1.51 | 4.19 |
| Liver weight | 466 | 393 | 331 |
| Portal blood flow | | | |
| Calc. liters in 30 min.* | 7.0 | 5.9 | 5.0 |
| Required for portal conc.†P* | 17.7 | 14.3 | 14.7 |
| Required for portal-jugular difference | 52.2 | 59.6 | 22.7 |
| Intestinal contents | | | |
| Found per cent dose/gram | 0.149 | 0.102 | 0.047 |
| Dilution required for portal P* | 4.4 | 6.2 | 14.4 |
| Dilution required for portal-jugular difference | 6.0 | 9.0 | 17.0 |

* Average value for portal blood flow to liver—0.5 cc./gram liver/min. (Grab, Janssen, and Rein, 1929).

† Assumption made that P* was not carried away by blood stream remained in lumen of gut.

strengthened our conclusion that considerable amounts of P* must be held in the intestinal mucosa.

In two additional dogs which were studied under comparable conditions, the intestine was removed from the point of cannulation to the junction with the large bowel; samples of mucosa, muscle, and whole tissue were taken for radioactivity determinations. The intestine as a whole was thus found to contain about 20 per cent of the dose, $\frac{9}{10}$ of which was held in the mucosa. At the end of 4 hours, therefore, $\frac{1}{5}$ of the administered dose is held in the intestinal tissue, principally the mucosa. Our data give us no indication of whether the phosphorus is simply trapped in the mucosa in the form in which it was given, inorganic phosphate, or whether it has replaced unlabeled phosphorus and become an integral part of the tissue.

SUMMARY

1. A study was made of the distribution of radioactive phosphorus, introduced directly into the duodenum of the dog, during the four hours immediately following the administration of the isotope.

2. Labeled phosphorus appeared in the general circulation within five minutes. The maximum change in the P* content of the portal and jugular blood occurred within the first half-hour; the portal P* level exceeded that of the jugular and carotid blood significantly for 3 to 3½ hours.

3. The specific activity of the liver rose rapidly for 2½ hours, after which it remained fairly constant.

4. At the end of 4 hours, between 0.4 and 5 per cent of the dose could be washed out of the small intestine and the intestinal tissue was found to contain 20 per cent of the dose—$\frac{9}{10}$ of which was in the mucosa.

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# AN EXPERIMENTAL STUDY OF THE TOURNIQUET AS A METHOD FOR INDUCING CIRCULATORY FAILURE IN THE DOG

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Tourniquets have long been used for temporary control of hemorrhage from vessels of the extremities, but only as an emergency measure, for it is well known that their subsequent release may lead to fatal shock. During World War I it was considered probable that the circulatory failure so induced was due to the absorption of toxic metabolites into the general circulation. This work has been adequately reviewed by Cannon (1). More recently several investigators have studied this shock inducing procedure (2–6)[2], without, however, reaching unanimous agreement as to the basic changes which lead to the circulatory collapse. Our attention was directed to a study of the tourniquet method for producing shock because of its apparent simplicity and the ease with which it could be quantitated.

METHODS EMPLOYED. Healthy, vigorous dogs weighing 8 to 20 kgm. were used. They were anesthetized with Nembutal (0.5 cc. per kgm. body weight, given intravenously), the hair carefully removed from both hind legs, and a heavy walled rubber tubing (diameter 120 mm.) was looped three times around each hip, as high as possible and tied very tightly. At the end of five hours the tourniquets were released.

Hematocrit determinations were made in capillary tubes centrifuged at 20,000 RPM in an air turbine; hemoglobin by the method of Newcomer; serum proteins by the falling drop method of Barbour and Hamilton (7); plasma volume by the T-1824 dye method of Gregersen and Stewart (8), using a spectrophotometer; blood pressure by the intra-arterial needle puncture method (9). Since both hind legs were involved in the experiment, the femoral artery usually was not available for frequent pressure recordings. Where pressure changes were believed critical, the brachial artery was exposed for this purpose. This was not adopted as a routine procedure because of the added trauma involved.

In these studies plasma volume changes, as measured by repeated dye injections, and as calculated on the basis of hematocrit and hemoglobin values proved to be of the same order of magnitude. For continuous records, the hematocrit, the simplest of the measurements, was used as the gauge of circulating plasma volume changes. The agreement between hematocrit change and alteration of

[1] Upjohn Research Fellow.

[2] Best and co-workers of Toronto University, and Winternitz of Yale University have also studied this problem, using dogs as experimental material (personal communication).

plasma volume was checked at intervals on a selected number of animals from each series by the direct dye injection method.

Anesthesia was not maintained longer than 24 hours. Ninety-six per cent of our untreated animals subjected to a 5 hour constriction of both legs died in shock. A lesser interval of constriction gave variable results, e.g., 50 per cent of the dogs survived a constriction of both legs for 3.5 hours, and none developed circulatory failure following release of the tubes for 2 hours. Substitution of rubber blood pressure cuffs inflated to 300 mm. Hg pressure for 5 hours proved fatal in only 50 per cent of the cases.

*Symptoms, hemoconcentration, etc., during the 5 hour constriction.* Blood pressure, pulse and respiration remained essentially unchanged throughout the interval during which the tourniquets were in place (table 2). There was, during this period, a progressive diminution in circulating plasma volume, as indicated by hematocrit, hemoglobin, serum protein, and plasma volume changes (table

TABLE 1

*Blood concentration changes in dogs subjected to a five hour period of constriction of both hind legs*

| | NO. OF DOGS | HEMATOCRIT | NO. OF DOGS | HEMOGLOBIN | NO. OF DOGS | SERUM PROTEIN | NO. OF DOGS | PLASMA VOLUME |
|---|---|---|---|---|---|---|---|---|
| | | *per cent* | | *grams per cent* | | *grams per cent* | | *cc./kgm.* |
| Initial | 40 | 38.3 ±0.5 | 40 | 15.1 ±0.3 | 32 | 5.7 ±0.1 | 12 | 49.7 ±0.8 |
| 2.5 hours | 11 | 47.4 ±1.7 | 11 | 18.5 ±0.7 | 11 | 6.6 ±0.2 | 6 | 46.3 ±0.9 |
| 5 hours | 40 | 48.8 ±0.7 | 40 | 19.5 ±0.5 | 32 | 6.5 ±0.3 | 12 | 44.3 ±0.8 |
| Release of constrictions | | | | | | | | |
| 6 hours | 22 | 64.2 ±1.5 | 24 | 28.0 ±0.6 | 18 | 7.6 ±0.3 | 8 | 30.4 ±1.5 |
| 8 hours | 19 | 69.7 ±1.3 | 22 | 29.6 ±0.7 | 15 | 7.2 ±0.3 | 6 | 26.0 ±1.6 |
| 11 hours | 10 | 70.9 ±1.7 | 14 | 29.5 ±0.6 | 11 | 7.1 ±0.2 | 6 | 25.4 ±1.3 |

1). Plasma volume determinations made prior to and after the placing of the tourniquets indicated that from 11 to 16 per cent of the original plasma volume had been trapped in the legs below the constrictions. For the calculation of plasma volume changes during the constriction period, therefore, hematocrit, hemoglobin and serum protein concentrations were corrected by 12 per cent. These calculations would indicate an average plasma volume reduction of some 10 per cent during the interval in which the tourniquets were in place. The greatest part of the plasma volume reduction occurs in the first 1 to 2 hours, with but slow decline thereafter. Amputation of the leg beyond the constrictors showed no observable leakage of blood. Therefore the plasma loss is seemingly due to a generalized transudation.

When the constrictors were removed, the immediate swelling of the legs indicated a rapid loss in plasma volume through the damaged capillaries. The hematocrit level rose rapidly to 60 to 68 per cent during the first hour, then more gradually for the next 2 to 3 hours, reaching a plateau at an average of 70.9

per cent (table 1). In the same interval, hemoglobin readings rose to 29.5 grams per cent, the blood coagulation time fell from an initial of about 6 minutes to from 1 to 2 minutes, and the plasma volume (T-1824) declined to 25.4 cc. per kgm. body weight. These values remained essentially unchanged until the death of the animal. In other words, of the total plasma volume reduction of 49 per cent during the application of the constrictors and their release, roughly a fourth was lost before untying the constrictions, a half was lost in the first hour after their release, and the final fourth in the following 2 to 3 hours.

Serum protein concentrations did not show changes of the order expected from the hematocrit, hemoglobin, and plasma volume values. A rise to 7.6 grams per cent followed the release of the constriction, but there was usually no further increase as the blood concentration became more severe. In fact, almost all animals showed a decline some time before death. This probably indicates a heavy loss of plasma protein into the extra-vascular regions of the injured legs greater than that which could be returned by the lymph channels. The clear yellow fluid expressed from the leg at death showed protein concentrations approximating that of plasma. Peritoneal fluid also showed a high protein content.

The extreme and rapid concentration of the blood immediately following the release of the constrictions was not necessarily accompanied by changes in clinical signs. Deep shock followed the removal by 3 to 4 hours, as judged by decline in skin temperature, pallid lips and a lessening of the need for anesthesia. The blood pressure declined to about 90 mm. Hg shortly after removal of the constrictions, but then fell only gradually to 75 or 80 mm. Hg, where it remained until shortly before death (table 2). Blood pressure changes therefore gave little clue as to the severity of the shock or of the approach of death. The pulse rate remained high, and was extremely variable, until a few hours before death, when there sometimes was a decline. The veins collapsed relatively early. About an hour before death, rapid gasping respirations were usually observed, and the heart beat became extremely irregular.

The survival periods of 25 dogs ranged from 3 to 27 hours after the release of the constrictions. One dog recovered (table 2). No correlation has been observed between the degree or the rapidity of hemoconcentration and the survival period of the animal.

*Autopsy findings in untreated animals.* Autopsy revealed few distinctive abnormalities. There was a slight congestion of the intestine but no evidence of hemorrhage; some congestion of the spleen, kidneys and liver; marked contraction of the spleen with raised areas on its surface; minor hemorrhages, sometimes widespread, into the adrenal cortex. Very little fluid was present in the peritoneal cavity.

In the earlier experiments about 60 per cent of the animals developed subcutaneous gas pockets in the tissues of the hind legs, and crepitation was felt. When the experimental technique was modified so that the legs were carefully washed and treated frequently with alcohol, during the constriction period, these infections became rare.

The hind limbs were swollen and filled with fluid which could be occasionally

expressed as either a straw colored or red tinged plasma like material. This was especially noticeable when transfusions were given immediately following release of the tourniquets.

*Effect of bandaging the limbs following release of the tourniquets.* The experimental evidence strongly indicated that the cause of death following release of the constrictions had origin in the local loss of fluid into the injured legs. An effort was therefore made to curtail this fluid loss by bandaging the legs snugly from thigh to toe with several layers of sterile gauze, followed by a tight wrapping

TABLE 2

*The effect of leg bandaging and of saline and plasma injections in the prevention of shock after a five hour constriction of both hind legs in the dog*

| NO. OF DOGS | BODY WEIGHT | INITIAL | | END OF CONSTRICTION | | 4–8 HOURS "SHOCK" | | SURVIVAL | PER CENT OF DOGS SURVIVING |
|---|---|---|---|---|---|---|---|---|---|
| | | Blood pressure | Pulse per minute | Blood pressure | Pulse per minute | Blood pressure | Pulse per minute | | |
| **Untreated controls** | | | | | | | | | |
| | *kgm.* | *mm. Hg* | | *mm. Hg* | | *mm. Hg* | | *hours* | |
| 25 | 12.8 | 111 ±1.4 | 128 ±4.0 | 108 ±1.5 | 163 ±2.4 | 78 ±1.5 | 175 ±2.2 | 8 ±0.7 | 4 |
| **Injured legs bandaged at the end of the constriction period** | | | | | | | | | |
| 22 | 12.5 | 114 ±1.3 | 132 ±3.6 | 113 ±1.3 | 169 ±2.2 | 110 ±1.3 | 172 ±2.0 | | 100 |
| **Saline infusion (300 and 900 cc.) following release of constrictions** | | | | | | | | | |
| 2 | 9.8 | 118 | 111 | 124 | 157 | 77 | 190 | 12 | 0 |
| **Plasma transfusion (25 cc./kgm.) given immediately upon release of the constrictions** | | | | | | | | | |
| 6 | 10.2 | 115 ±1.6 | 143 ±3.2 | 102 ±2.0 | 164 ±3.0 | 83 ±1.6 | 148 ±2.5 | 8 ±0.5 | 17 |
| **Plasma transfusion (25 cc./kgm.) given 3 hours after release of the constrictions** | | | | | | | | | |
| 6 | 10.3 | 118 ±1.4 | 140 ±3.0 | 95 ±2.0 | 162 ±2.4 | 75 ±1.4 | 151 ±3.0 | 12 ±1.0 | 33 |
| **Plasma transfusion (25 cc./kgm.) given in 5 equal doses: at time of release of constrictions, and at 1, 2, 4 and 7 hours later** | | | | | | | | | |
| 7 | 10.6 | 112 ±1.6 | 111 ±3.6 | 106 ±1.5 | 162 ±2.3 | 105 ±1.1 | 174 ±2.0 | | 100 |

with adhesive tape. Despite the tapes, some hemoconcentration still occurred (fig. 1). The average plasma volume reduction over that reached in the constriction period was 10 cc. per kgm. body weight, or 20 per cent (fig. 1).

The animals with bandaged legs exhibited no signs of shock. They were alert from the time they were allowed to recover from the anesthetic (24 hrs.) and took food and water at that time (table 2). However, the hemoconcentration was relieved slowly, especially before water was taken (fig. 1). After 48 to 72 hours the hematocrit and hemoglobin concentrations stabilized at a

level usually somewhat higher than the initial, pre-experimental, one which was maintained until the wrappings were removed. These results are somewhat comparable to those of Duncan and Blalock (10).

Despite the fact that the bandaged dogs presented no evidence whatever of shock, any attempt to remove the bandages before 24 hours was followed by circulatory collapse. The limbs swelled, the blood concentrated and the animals

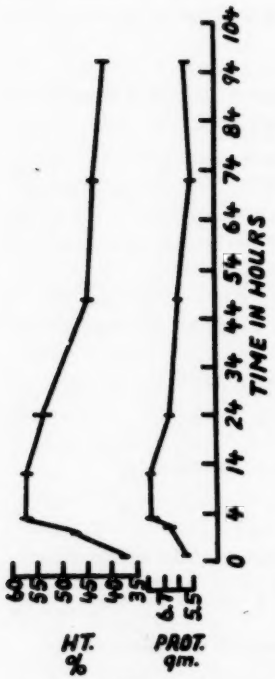


Fig. 1

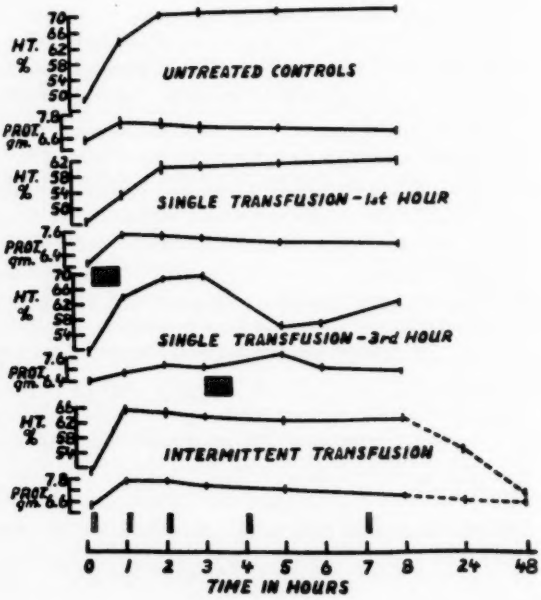
HT. %
PROT. gm.
UNTREATED CONTROLS
SINGLE TRANSFUSION - 1st HOUR
SINGLE TRANSFUSION - 3rd HOUR
INTERMITTENT TRANSFUSION
0 1 2 3 4 5 6 7 8 24 48
TIME IN HOURS

Fig. 2

Fig. 1. Blood concentration and dilution changes following release of the constrictions (zero time), while the injured legs are enclosed in tight bandages.

Fig. 2. Hematocrit and serum protein changes following plasma transfusions after a five hour constriction of both hind legs. Blocks represent amount and time of transfusions.

Single transfusion: 25 cc. heparinized plasma per kilogram of body weight.

Intermittent transfusion: Five injections of 5 cc. per kilogram of body weight each, heparinized plasma.

presented the same symptoms as control animals did when the constrictions were released. The bandages could usually be removed after 24 hours without jeopardizing the life of the animal (table 3). However, some concentration followed in practically all cases.

During the period in which the bandages were in place, blood flow in the injured limb seemed well established. At least, the dye T-1824 injected into the femoral artery or saphenous vein could be recovered in the jugular or fore limb

veins within 30 seconds. Although circulatory failure was prevented by bandaging the legs for at least 24 hours after release of the constrictions, the actual recovery of the legs was a slow process. Paralysis was present over several weeks. Necrotic areas appeared in the region where the constrictions had been placed with an eventual skin sloughing and raw ulcers. Severe infection often developed unless appropriate precautionary measures were taken.

TABLE 3

*Blood concentration changes following removal of the bandages from the injured legs*

| DOG | BANDAGES REMOVED | INITIAL | | | PRIOR BANDAGE REMOVAL | | | 5 HRS. AFTER REMOVAL | | | 24 HRS. AFTER REMOVAL | | | SURVIVAL HOURS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hematocrit | Hemoglobin | Serum protein | Hematocrit | Hemoglobin | Serum protein | Hematocrit | Hemoglobin | Serum protein | Hematocrit | Hemoglobin | Serum protein | |
| | *days* | *per cent* | *grams per cent* | *grams per cent* | *per cent* | *grams per cent* | *grams per cent* | *per cent* | *grams per cent* | *grams per cent* | *per cent* | *grams per cent* | *grams per cent* | |
| 1 | ½ | 47.9 | 19.8 | 6.8 | 67.2 | 28.8 | 7.2 | 80.0 | 33.0 | 7.0 | | | | 5 |
| 2 | ½ | 52.0 | 23.4 | 6.4 | 63.3 | 30.4 | 7.4 | 81.6 | 33.0 | 8.7 | | | | 4 |
| 3 | 1 | 42.5 | 19.5 | 6.5 | 63.8 | 28.8 | 7.4 | 65.1 | 29.9 | 6.8 | 58.4 | 27.4 | 5.1 | Recovered |
| 4 | 1 | 32.4 | 15.2 | 5.2 | 50.3 | 21.7 | 6.2 | 56.0 | 29.9 | 6.8 | 51.4 | 20.9 | 5.3 | Recovered |
| 5 | 2 | 41.8 | 15.1 | 6.8 | 49.1 | 17.3 | 7.1 | 54.0 | 19.0 | 7.3 | | | | 5 |
| 6 | 2 | 35.5 | 11.2 | 6.2 | 40.0 | 13.3 | 6.9 | 49.0 | 16.0 | 7.4 | | | | 10 |
| 7 | 2 | 40.3 | 15.7 | 4.9 | 49.2 | 23.8 | 6.1 | 54.1 | 24.5 | 7.8 | 47.5 | 20.5 | 4.3 | Recovered |
| 8 | 2 | 43.2 | 16.0 | 6.1 | 50.0 | 24.0 | 6.4 | 48.0 | 21.4 | 6.1 | 47.5 | 21.6 | 5.9 | Recovered |
| 9 | 3 | 41.0 | 14.2 | 5.4 | 44.6 | 18.9 | 7.8 | 62.8 | 21.2 | 7.9 | | | | 12 |
| 10 | 3 | 29.6 | 11.7 | 5.3 | 40.4 | 14.2 | 4.9 | 43.6 | 16.7 | 4.8 | 39.5 | 16.0 | 4.0 | Recovered |
| 11 | 3 | 41.5 | 11.9 | 4.9 | 38.8 | 14.9 | 5.4 | 51.6 | 22.0 | 4.8 | 50.0 | 21.0 | 4.4 | Recovered |
| 12 | 4 | 38.0 | 15.7 | 4.7 | 36.3 | 13.9 | 5.0 | 34.9 | 12.7 | 5.5 | 34.0 | 12.6 | 5.2 | Recovered |
| 13 | 5 | 31.6 | 13.8 | 4.8 | 36.1 | 16.2 | 6.0 | 40.2 | 16.4 | 6.0 | 39.1 | 16.2 | 5.4 | Recovered |
| 14 | 5 | 39.6 | 17.3 | 6.3 | 37.5 | 15.0 | 6.5 | 38.0 | 15.2 | 6.2 | 37.8 | 15.0 | 5.7 | Recovered |
| 15 | 5 | 44.0 | 20.0 | 6.2 | 47.0 | 16.7 | 5.8 | 49.8 | 19.0 | 6.0 | 49.2 | 18.7 | 6.0 | Recovered |
| 16* | 5 | 37.2 | 12.4 | 6.2 | 34.2 | 13.6 | 5.7 | 42.0 | 18.7 | 6.0 | | | | Tapes replaced |
| 17* | 5 | 38.8 | 15.2 | 5.6 | 37.8 | 15.5 | 5.8 | 47.0 | 19.0 | 7.2 | | | | Tapes replaced |
| 16 | 8 | | | | 31.4 | 15.6 | 5.7 | 35.1 | 17.7 | 6.6 | 34.2 | 15.5 | 5.7 | Recovered |
| 17 | 8 | | | | 38.3 | 18.3 | 5.4 | 38.5 | 18.2 | 5.2 | 30.2 | 15.1 | 4.5 | Recovered |
| Ave...19 | | 39.8 | 15.1 | 5.8 | 45.0 | 18.4 | 6.2 | 51.6 | 21.2 | 6.5 | 43.2 | 18.4 | 4.8 | |

* Bandaged legs placed in ice bath for 48 hours (see text).

Bacterial infection, observed frequently in the first non bandaged control animals, developed within 24 to 36 hours in the bandaged legs. Extensive accumulations of gas appeared in the regions of the thighs and lower abdomen, with the animal rarely surviving more than 24 hours after their first appearance. Smears made of the serous exudate from the gas filled tissues of a badly infected dog showed at least four different groups of micro-organisms. Further cultural investigation under aerobic and anaerobic conditions revealed the presence of *a*, Clostridium welchii; *b*, Clostridium sporogenes; *c*, Streptococcus putridus, and

*d* B. coli.[3] Wilson and Roome (5) reported the presence of an unidentified anaerobic gas forming bacillus in the constricted legs of 8 of 11 dogs. They recovered the same or a closely similar organism from the normal legs of 7 of 11 animals.

To reduce the incidence of these infections, the limbs of the dogs were closely clipped with an electric clipper and the skin of the legs and toes thoroughly washed with green soap. A solution of picric acid was then applied, followed by 80 per cent alcohol. After the constrictions were placed, the legs were lightly covered with towels which were kept soaked with alcohol. Bandages placed on the legs after release of the tourniquets were moistened with alcohol and kept wet as long as they were on the limbs. This procedure, while tedious, did successfully prevent infection, showing that the probable source of the organisms producing the infection was the skin of the devitalized limbs.

Allen (6) has stressed the point that lowering the temperature of the ligated limb reduces the danger of shock and subsequent gangrene, and enables the affected part to tolerate lack of circulation for a longer time. The ameliorating effect of chilling has been attributed to a depression of tissue metabolism and reduced formation of toxic substances in the ligated extremities. Our experience with reduced temperature has been confined to packing the bandaged limbs in ice after release of the constrictions, merely as a means of retarding bacterial growth. In this regard the use of ice was successful, but the procedure was so cumbersome that it had no advantage over keeping the bandages wet with alcohol. Furthermore, the ice treatment seemed definitely to retard the rehabilitation of the vessels of the injured legs, so that a longer interval was required before the wrappings could safely be removed (table 3).

*Effect of saline injections and plasma transfusions.* Wilson and Roome (5) reported that transfusions of large amounts of citrated plasma and saline were ineffective. The administered fluid failed to remain in the general circulation but was lost into the tissues of the damaged limbs. Allen (6) states that early and abundant injections of saline allowed recovery when injections of blood or plasma failed to save life.

In our hands saline infusions, even in large quantity, were without effect (table 2). It did not matter whether the salt was given immediately following release of the constrictions, or later. The hemoconcentration was not relieved and the legs showed massive edema. The rate of leg swelling seemed directly correlated with the amount of saline administered and the rate at which it was given.

*Plasma transfusions.* These were of two types: 1, continuous, and 2, intermittent. The efficacy of the transfusion was found to depend upon the manner in which it was given. In both types the total quantity of plasma was the same, as calculated on a body weight basis.

A. *Continuous transfusion given immediately following release of the tourniquets.* The most logical time for the administration of a transfusion would seem to be

[3] We are indebted to Mr. George Warren of the Section of Microbiology for identifying these organisms.

immediately following the release of the constrictions, or just before the animal entered the period of shock. Transfusions were therefore begun within 15 minutes of the release of the constrictions and continued over a period of 30 to 50 minutes. This treatment failed to relieve the hemoconcentration (fig. 2). Nor did the transfusion serve to prevent or even postpone death (table 2). The fact that the legs continued to swell, that the hemoconcentration became progressively more severe, and that the serum protein concentration declined steadily from the high level reached immediately following the transfusion, makes it evident that the administered plasma failed to remain in the circulation but leaked into the leg tissues.

Of 6 animals given plasma transfusions in this manner, only 1 survived (table 2). This dog failed to exhibit a typical degree of hemoconcentration even in the pre-transfusion period.

B. *Continuous transfusion given three hours after release of the tourniquets.* Untreated control animals showed the greatest part of the total plasma loss immediately after removal of the constrictions. A transfusion given during this period of rapid leakage not only did not reduce this loss, but the plasma administered was also lost from the blood stream. It is conceivable, therefore, that the injected fluid, by increasing the fluid transfer through the injured capillary bed, was actually detrimental to the animal. On the other hand, a transfusion given after this initial plasma volume depletion, even though the animal was now in shock, might prove more efficacious. To check this possibility, continuous transfusions were given a second series of animals after an interval of three hours had elapsed since removal of the constrictions. At this time the maximum changes in hemoconcentration had occurred (fig. 2) and early shock symptoms were present.

The response to such delayed transfusions was better than that to the transfusion given immediately upon release of the constrictions, but the number of animals which recovered was small (table 2).

C. *Intermittent transfusions of small amounts of plasma.* If instead of giving single transfusions over 30 minutes, the total amount of plasma was divided into five equal portions and transfused at the time of release of the constrictions, and at the end of the first, second, fourth, and sixth hours thereafter, the effect in preventing shock was truly dramatic. The animals all recovered and ate within a few hours of recovery from the anesthesia. Unlike the bandaged animals, they showed no gangrene after several days. The legs were paralyzed, but otherwise the animals remained in perfect health and could be kept as long as desired.

The clue to the dramatic response to the intermittent transfusion is not afforded by a survey of the hemoconcentration and plasma volume changes (fig. 2). The data indicate that small intermittent transfusions are not retained in the blood stream any more readily than is the continuous transfusion. Nor is the absolute level of hemoconcentration different from that of either series of animals receiving the continuous transfusion.

The critical factor allowing survival is not merely the ability to dilute the

blood or lower the hemoconcentration, for dilution progresses but slowly in the animals of all series. Complete restoration to the normal hematocrit levels in intermittently transfused animals, is rarely complete by the 48th hour, even though the animal is in comparatively good health. The evidence is suggestive therefore that the striking beneficial effects of small amounts of plasma given intermittently cannot be fully accounted for by a mere physical dilution of the circulating blood volume.

Discussion. The rapid hemoconcentration, coinciding with marked swelling of the legs, indicated that undue transudation of plasma through the injured capillaries into the legs, with a consequent sharp reduction in circulating blood volume, is the initiating factor in the shock which follows release of the constrictions. The fact that merely tightly bandaging the limbs immediately after release of the constrictions will prevent the circulatory failure, seems strong evidence for this conclusion. It is not to be inferred, however, that death is due solely to the extreme hemoconcentration and reduction in circulating blood volume, for hematocrit levels fully as high may be reached in experimental animals (e.g., with intraperitoneal glucose injections) without leading to a fatal issue. Also, the restorative action of the intermittent transfusion cannot be correlated with hemodilution changes.

It has been assumed by some that the actual shock following release of constrictions on the extremities is due to a flooding of the systemic circulation with toxic products from the injured area. The present experiments present some evidence against this view: 1. The circulation of the bandaged limb is patent at all times, as shown by the recovery of dye injected into the leg veins. Any toxin present in the legs should therefore have free access to the general circulation. Yet bandaged animals exhibit no signs of shock. 2. Removal of the bandages within the first 24 hours is followed by swelling of the legs, hemoconcentration and death. Replacement of the bandages, if done promptly, before the hemoconcentration has become well advanced, will promptly stop further leg swelling and hemoconcentration and allow normal recovery of the animal. The longer the interval allowed before the bandages are removed, the less the subsequent hemoconcentration and the more rapid the recovery of the animal from the temporary concentration. Apparently the minute vessels of the limbs, if given sufficient time, gradually recover to the point where excessive plasma leakage does not occur. The ability of the intermittent transfusion to prevent shock in an animal without bandages or other means of preventing local fluid loss, without inducing an appreciable hemodilution, is not clear. Further experiments are in progress to attempt a more complete explanation of this point.

## SUMMARY

1. The tourniquet method for producing shock, in which both hind legs were constricted by heavy walled rubber tubing tightly tied around the hips for a period of 5 hours, has been studied in a large series of dogs.

2. Of 25 untreated control animals, all but one died in shock following the release of the constrictions. The survival periods ranged from 3 to 27 hours.

Associated with the shock condition was a marked swelling of the injured legs, an intense hemoconcentration, and a plasma volume reduction of 49 per cent.

3. In all animals in which leg infections were prevented by proper antiseptic treatment, the snug bandaging of both legs prior to, or immediately after removal of the constrictions, successfully prevented shock. While shock was prevented by the bandaging, the legs were paralyzed and recovered but slowly.

4. A plasma transfusion of 25 cc. per kgm. body weight, given immediately after release of the constrictions or later, failed to prevent the hemoconcentration, and did not prevent death in 9 of 12 dogs.

5. The same amount of plasma, divided into five doses of 5 cc. per kgm. body weight each, and transfused intermittently over a seven hour period, prevented shock in all of 7 dogs. This positive effect could not be correlated with hemodilution changes.

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# CARDIAC INJURY POTENTIALS[1]

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A localized region of injury of cardiac muscle, electrically negative when the heart is at rest, becomes electrically positive, with respect to the potential of resting uninjured muscle, when the muscle contiguous to the injury enters into activity (1) (2) (3). The present report is concerned with certain characteristics of this phenomenon, particularly its development when injury is produced and its relation to the contraction of heart muscle.

METHODS. For the production of localized injury of surface regions on the heart and the recording of the resulting injury potentials, a suction electrode of the type first described by H. C. Wiggers (4) was employed in all experiments. A small tab of heart muscle is sucked into a glass tube of 1 mm. internal diameter and makes contact with the wick of a zinc-sulphate electrode. In most cases the other electrode completing the circuit to the amplifier input was placed in contact with a hind leg of the animal (unipolar recording). The amplifiers used in connection with the cathode ray oscillographs are balanced, two channel, direct coupled and entirely free from detectable amplifier drift at amplifications considerably higher than those used in the experiments. Figure 1A shows the response to a potential change in the input circuit of 1 mv.

In the experiments concerned with intraventricular pressure, a membrane manometer provided with a photo-electric cell was used (5). All measurements from the curves were made with a micrometer comparator. Most of the experiments were carried out on the exposed hearts of large specimens of the snapping turtle (Chelydra Serpentina) after destruction of the brain and spinal cord and on the exposed hearts of dogs under ether or nembutal anesthesia.

*Characteristics and development of the injury potential.* The injury potential-time curves from the heart recorded by the use of the suction electrode are remarkably consistent in their contour in the same and even in widely different animal species. Figure 1 is a record resulting from suction applied to the exposed heart of Limulus. In this, as in all records, unless noted otherwise, the recording is unipolar. Downstroke in all records is in the direction of positive potential at the heart contact. The principal differences from the injury potential of Limulus and similar potentials recorded from the heart of the carp (fig. 2) and from the hearts of turtles and dogs (figs. 3 to 7) are in the more rapid decline in potential following the positive maximum and in the presence of superposed oscillations on the declining limb.

One of the most interesting features of the injury potential is its localized character. When a unipolar lead is brought along the surface of the heart successively nearer to a region of injury, no trace of the injury potential is evident

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

until the contact is within about a millimeter from the margin of the injury. Another indication of this localization is the fact that the curve of an extrasystole differs not at all or only in minor ways from the curve of a normal heart cycle. Figure 3 is a record of an extrasystole in the turtle's ventricle produced by a condensor discharge shock applied at some distance from the suction electrode. In the extrasystolic cycle (third cycle) the time rate of change in voltage in passing from the state of maximum negativity to the state of maximum positivity is somewhat less than in the normal cycle. Figure 4 is a similar record from a dog's ventricle. In this the maximum rate of voltage change, in contrast to the turtle record, is slightly greater in the extrasystolic cycle than in the normal cycle. The localized character of the injury potential is no doubt dependent on the nature of the potential distribution in the form of concentric rings of positive and negative charges (1) (2). If the seat of injury is pictured as a localized cup shaped depression in the muscle with this kind of charge distribution, there is to be expected no resulting potential difference on the heart surface. On the other hand, if the heart containing an injured region is surrounded by an electrically conducting field, as it is in the intact animal, a potential field would be established in which potentials would be apparent at all points at which the solid angle subtended by the injury is not zero and of a magnitude proportional to this angle. We have proven this to be true experimentally by immersing an isolated heart containing an injured region in a saline bath and plotting the potential field around it. It would seem clear that it is the potential field resulting from injury potentials in an ischemic region of heart muscle which is responsible for the characteristic electrocardiographic changes which occur in this condition. An ischemic region of the dog's ventricle develops injury potentials which resemble those produced by other forms of injury.

It should also be clearly recognized that with the heart in situ, any experimental procedure which produces injury to the heart surface will cause modifications in electrocardiograms which result directly from the injury potentials developing in the region so injured. Efforts to determine the contribution to the normal electrocardiogram of particular regions of the heart by attempting to prevent normal action potential development from other regions by injuring the latter (6), merely results in the replacement of the normal action potentials by injury potentials. Since it is obviously impossible to ablate any region without producing injury, and since injury of any type produces large and sustained injury activity potentials which directly modify the electrocardiogram, no conclusions can be drawn as to the normal distribution of potentials from experimental procedures of this type.

Injury potentials develop fully within one or two cycles following the application of suction to a surface region of the turtle heart. If the suction is started during systole, the initial potential change is in the positive direction and to an amount which is approximately the same as that which occurs at the same point in the cardiac cycle in subsequent cycles. In figure 5 two examples are given of the development of injury potentials resulting from the application of suction

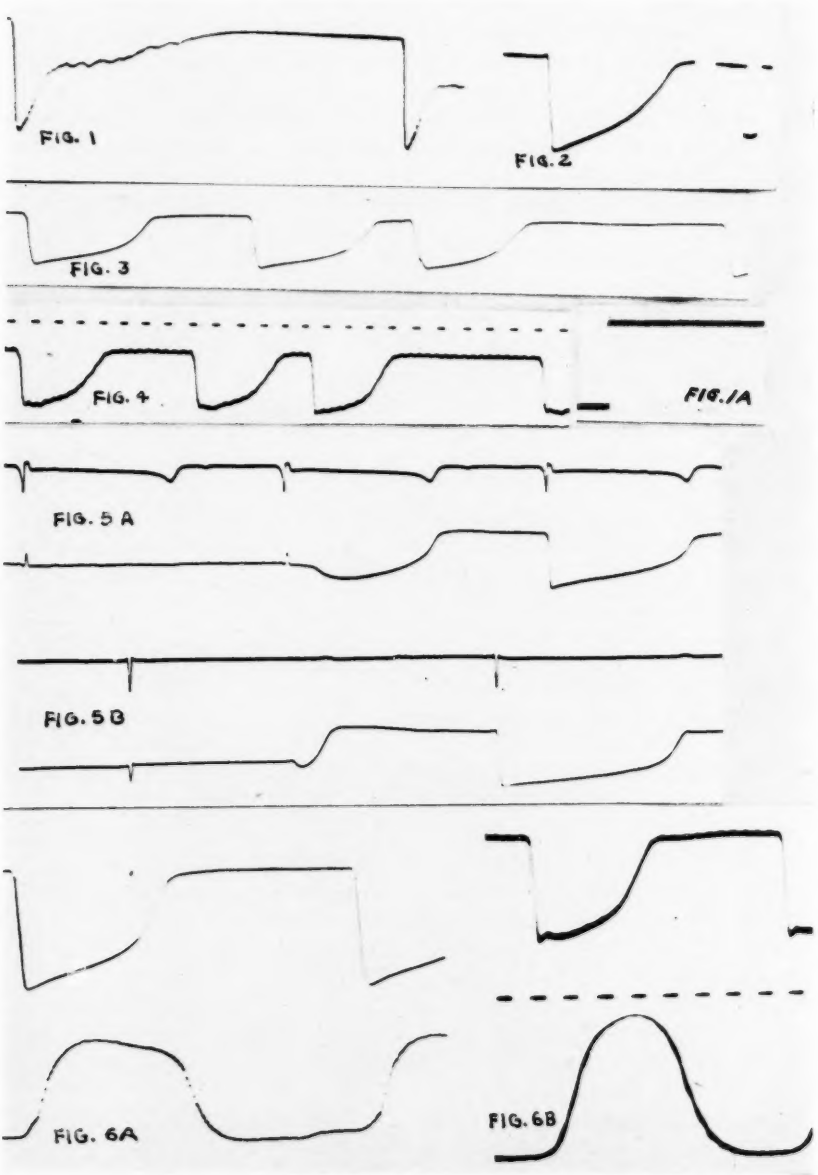



Fig. 1A. Response of one of the amplifier-oscillograph units to a change of potential in the input circuit of 1 mv.

Fig. 1. An injury potential-time curve recorded from the exposed heart of Limulus.

Fig. 2. A similar curve as in the preceding figure from the exposed heart of a carp. The

to local regions of the turtle's ventricle. The upper records in each case are reference curves, the lower curves are unipolar leads from the suction electrode. In A, suction was started early in systole in the second cycle shown. A positive potential developed which reached a maximum value of 11.1 mv. Before the end of systole, as indicated by the T wave on the reference curve, the positive potential began to decline and the region later became the seat of a negative potential which reached a maximum value of 20.8 mv. during the diastolic period. With the onset of the following systole, the potential sign reversed rapidly to reach a positive potential maximum of 18.6 mv. In B, the suction was started considerably later in the systolic period. The first change in potential was again in the positive direction, but the maximum positive potential reached in this cycle was only 4.0 mv. The potential then reversed in sign to reach a maximum negative potential of 24.4 mv. during diastole, followed by a maximum positive potential of 17.0 mv. early in the next cycle.

In the dog, injury potentials develop more slowly following the application of suction and may not be completely developed until after some ten or fifteen cycles.

*The time relations of ventricle injury potentials and intraventricular pressure.* Simultaneous recordings of injury potentials from various regions on the surface of the ventricle along with intraventricular pressure, show that the injury activity potential starts before the rise of pressure begins. The maximum positive potential falls within the initial period of slow rise of intraventricular pressure.

In figure 6 there are reproduced records of injury activity potentials and intraventricular pressure in the turtle and in the dog. In the turtle record (A), the injury activity potential starts 0.01 sec. before the start of the rise of pressure. The maximum positive potential and the end of the initial slow rise of pressure are coincident. The potential returns to the diastolic level 0.251 sec. before the end of the fall of intraventricular pressure. In the dog record (B)

calibration curve at the right in this figure records a difference of potential across the input of the amplifier of 20 mv.

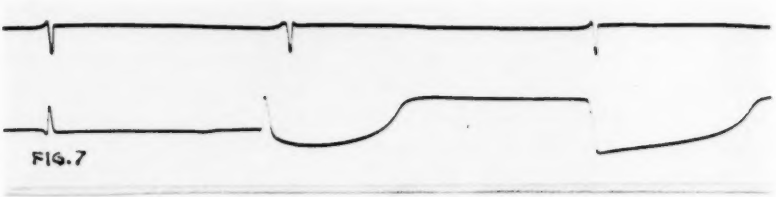
Fig. 3. Comparison of the injury potential-time curve in an extrasystolic cycle with normal cycles from the ventricle of a turtle. The first two cycles shown are normal and the third cycle is the extrasystole, followed by a compensatory pause. The extrasystole was caused by a brief condensor discharge applied to the base of the ventricle about 2.5 cm. distance from the injury. For further discussion see text. Speed of recording, 15 mm./sec.

Fig. 4. Similar record to that of figure 3 made from the anterior surface of the right ventricle of a dog. The extrasystole is the third cycle shown. For further discussion see text. Speed of recording, 69 mm./sec.

Fig. 5. The development of injury potential from the application of suction at different times in the cardiac cycle of the turtle ventricle. The upper curves in A and B are differential curves from the base of the ventricle and are used as reference curves. In A, suction was applied early, in B late in the systolic period. For further discussion see text. Speed of recording, 15 mm./sec.

Fig. 6. The upper curves are injury potential-time curves from a ventricle region, the lower curves intraventricular pressure. A is from a turtle, B from a dog. A rise of the lower curve indicates a rise of pressure. For further discussion see text. A was recorded at a speed of 20 mm./sec., B at a speed of 69 mm./sec.

the injury activity potential starts 0.008 sec. before the start of the pressure rise. The positive potential maximum occurs 0.014 sec. before the end of the initial



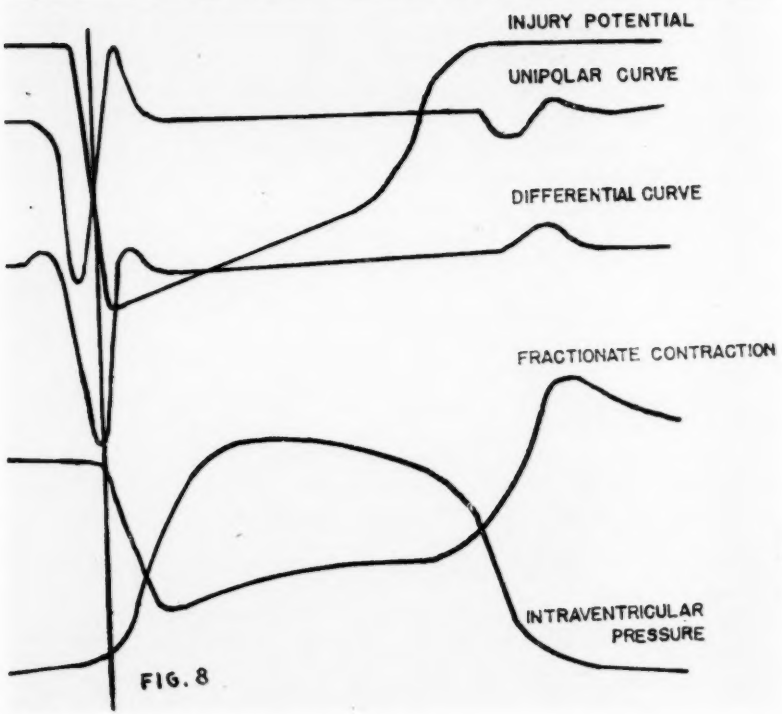




Fig. 7. Illustrates the type of record from which the onset of fractionate contraction in a region before injury is produced, is compared with the onset of the injury potential-time curve from the same region after injury is produced. The upper curve is a differential curve from the ventricular base and serves as a constant reference curve. The first cycle of the lower curve is a differential potential-time curve from a region near the midventricle to which suction was applied and injury produced near the end of this cycle. This record was made from a turtle. For further discussion see text. Speed of recording, 15 mm./sec.

Fig. 8. Comparison of the time relations of certain electrical and mechanical activities of the heart. For discussion, see text.

slow rise of pressure and the potential returns to diastolic level 0.099 sec. before the end of the fall of intraventricular pressure.

*The relation of the injury activity potential to the onset of fractionate contraction of surface regions of the ventricle.* While it is most probable that an injured region of heart muscle does not contract, the muscle at the margin of the injury does, and the contraction, or some process associated with the contraction of the muscle immediately surrounding the region of injury is responsible for the production of the injury activity potential. It is therefore of especial interest to obtain, if possible, the time relations of the injury potential and the contraction of the muscle contiguous to the region of injury.

It has been recently shown (7) (8) that the onset of fractionate contraction at surface regions of the ventricles of the turtle and dog is coincident, within small errors of measurement, with the occurrence of the main peak of the differential potential-time curve recorded from the region. In an effort to relate the injury activity potential to contraction of the contiguous muscle, we have recorded differential curves from regions before injury and injury activity potentials from the same regions after suction injury was produced. For these experiments a suction electrode was altered to serve also as a differential electrode by mounting a wick electrode on the outside of the suction tube so that contact with the heart surface is made at the outside margin of the tube. When this electrode is applied to a surface region without suction, it represents two electrode contacts approximately a millimeter apart, sufficiently close to meet the requirements for a differential lead. After recording the differential curve along with a constant reference curve suction is applied and the injury activity potential recorded from the same region. The procedure is illustrated by figure 7 from an experiment on a turtle. The upper curve is from a differential lead and serves as a constant reference curve. The first cycle of the lower curve is a differential curve recorded by means of a suction electrode modified as described. Toward the end of this cycle suction was started. The lower curve moves in the direction of negative potential and then quickly reverses to a positive potential in the next systole. In the third cycle shown, the injury activity potential is fully developed. Measurements of these curves show that in the normal cycle preceding suction, the main differential peak precedes the peak of the reference curve by 0.0416 sec. In the cycle after the injury activity potential had developed, the onset of the potential change precedes the reference peak by 0.1260 sec. By taking a point 0.0416 sec. before the reference peak, this point falls approximately midway between the onset of the injury activity potential and its maximum when the voltage change in the positive direction has reached 20.2 mv. The total voltage change from the negative potential of rest to the maximum positive potential is 44.9 mv.

We have made measurements of this type from 69 records obtained from 8 turtle hearts and from 29 records from 2 dog hearts. In all cases it was found that the main differential peak, recorded before injury was produced, coincided in time with the period on the injury potential curve, recorded after injury, during which the potential is changing rapidly in the positive direction. From these results it is concluded that the injury activity potential starts in a region before fractionate contraction begins in the muscle contiguous to the injury

and that the onset of contraction in the surrounding muscle occurs at a time when the potential of the injured region is changing most rapidly from the electronegative state characteristic of rest to the electropositive state characteristic of activity. That the injury activity potential starts before contraction is also evident from the fact that its onset from all surface regions precedes the rise of intraventricular pressure, as noted in the preceding section.

Discussion. The time relations of electrical and mechanical events in the heart, as derived from the results of the present and preceding experiments (7) (8) are shown diagrammatically in figure 8. The curves shown were all redrawn from original records in which two of the curves were recorded simultaneously. The onset of fractionate contraction at the various surface regions of the ventricle occurs during the initial period of slow rise of intraventricular pressure. At any one region, the onset of contraction is coincident with the main peak of the differential curve and with the maximum time rate of change of the unipolar curve. The onset of the injury activity potential precedes these events by a definite interval.

The injury activity potential differs from other electrical potentials that may be obtained from the heart in that it *starts* at different time instants at different surface regions and that the start is definitely related to the onset of fractionate contraction in the particular region. The start of the injury activity potential precedes the onset of fractionate contraction at every region by an interval which is nearly constant, especially in the same heart. It would seem obvious that some changes are occurring in the muscle contiguous to the injured region which precede the contraction of the muscle and which are associated with a rapid change of potential of the region. The view that some such change occurs preceding contraction, commonly ascribed to the depolarization of a polarized membrane, has long been prevalent. This hypothetical process has been generally known as "the process of excitation" or "impulse" and has been identified with the occurrence of a local fall of electrical potential or "negativity." On the other hand, the two types of potential-time curves which may be employed to reveal the electrical state of the normal muscle, the unipolar and differential curves, clearly disprove the "negativity" hypothesis but show no feature which can be identified with the start of the excitation process.

In a preceding paper (1) it was postulated on theoretical grounds that the injury potentials are associated with concentric rings of positive and negative charges at the margin of the injury, which reverse in polarity when the surrounding muscle enters into activity. Because of the presence of an apparently new disposition of electrical charges and because of the large and prolonged potential change, which lasts throughout the whole period of contraction, we expressed the opinion that the injury potentials are the expression of new charge distributions that do not exist in uninjured regions. Sugarman, Katz and Jochim (2) constructed a model consisting of concentric metal rings placed in a saline bath and connected to a source of alternating current. They found that the potential distribution shown by this model was similar to that in a cardiac muscle injury. From these experiments the conclusion was drawn that the

injury potentials of the heart could be explained in terms of the theory of the polarized membrane, if one assumed that the injured region was in a constant state of partial depolarization. The alternation of the surrounding normal muscle from a state of maximum polarization to complete depolarization would account for the resting and activity injury potentials without the assumption of any new source of potential. Although this ingenious explanation is of interest it should be pointed out that with our present lack of knowledge as to many of the details of the electrical state in heart muscle associated with injury, particularly as regards electrical resistance relations, any models composed in part of low resistance metal conductors may be far from a true representation of the electrical state present in the heart. The observations quoted previously in the present paper regarding the electrical field established by a heart injury when immersed in a volume conductor would indicate that a model formed of concentric segments of two spheres of unequal radii would be perhaps a nearer approximation. Any conclusions drawn from results obtained by models supposed to represent the electrical conditions present in and around a cardiac injury, must, with our present meager knowledge, be considered with great reserve. The best model that we have of the distribution of injury potentials is the living preparation itself.

The marked constancy of the injury activity potential in form and magnitude from different regions of the heart and the fact that it differs to such a marked degree from normal action potentials recorded from the same regions before injury, makes it difficult to assume that it is entirely a representation of a process that occurs in uninjured muscle. The unipolar curves from different regions have characteristics which depend on the particular region, the potential of which may be initially positive or negative and undergo two or three reversals. The character of this curve is usually profoundly modified in an extrasystole produced by artificial stimulation. The contour and magnitude of the injury activity potential are essentially the same from all regions in normal beats and in extrasystoles. It seems most probable that it may be considered, in large part at least, as independent of the potential changes which occur under normal conditions in the surrounding muscle. It may well be, however, that the initial part of this potential change, the start of which precedes contraction by an approximately constant interval, signals some phenomenon which normally occurs in contiguous tissue before the mechanical process of contraction starts.

## SUMMARY

The potential time curve, derived from an injured region of heart muscle by the use of the suction electrode, is remarkably similar in contour and magnitude when recorded from different regions of the same heart, from different animals of the same species and even from animals widely separated in the animal scale. It represents a local potential change which does not involve the normal muscle contiguous to the injury, although a potential field develops in a conducting field which surrounds it on all sides. The contour and magnitude of the curves differ little in extrasystoles as compared with normal beats, in con-

trast to the marked differences in unipolar and differential potential time curves in these two circumstances.

The change in an injured region from a negative potential to a positive potential, which occurs when the muscle contiguous to the injury enters into activity, is due to some process in the contiguous uninjured muscle or at the boundary between the injured and uninjured muscle. The start of this potential change at all surface regions of the ventricle precedes the rise of intraventricular pressure by an interval which varies with the location of the injury. At each local region, its onset precedes the onset of local or fractionate contraction of the region by an approximately constant interval.

The possible relation of the start of the injury activity potential to the "excitation process" or "impulse" in the contiguous active muscle is discussed.

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# THE INFLUENCE OF GROWTH ON THE PHOSPHORUS METABOLISM OF THE MOUSE AND THE EFFECT OF THYROXINE AT VARIOUS AGES

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Although it has been known for some time that the phosphorus turnover of various tissues decreases with age, probably corresponding to the fall in basal metabolic rate with age, little specific data are available on this point. In this connection we have compared the phosphorus turnover of several tissues of the mouse at three age levels, namely, 6, 12 and 24 weeks.

Since thyroxine is known to cause a general increase in metabolism, it was of interest to determine the effect of the hormone on phosphorus metabolism. For this purpose we have compared the total phosphorus turnover of the various tissues of thyroxine-treated mice of these age levels with that of control animals.

METHODS. In this investigation, young albino mice of three age groups (6, 12 and 24 weeks) were used, having average weights of 11.5, 22.0 and 27.0 grams, respectively. They had been maintained on a diet of oats and Purina dog chow.

In each group the experimental animals received intraperitoneally on two successive days a dose of 0.05 mgm. of crystalline thyroxine (Roche-Organon) dissolved in 0.2 cc. of 0.01 N NaOH, while the control animals received 0.2 cc. of 0.01 N NaOH. All animals received intraperitoneally radioactive phosphorus in the form of $Na_2HPO_4$ 24 hours after the last thyroxine administration and were sacrificed by decapitation 24 hours after the injection of the labeled phosphorus.

The liver, brain, spleen, kidney, heart and tibia were removed for analysis; the blood was collected at decapitation. The tissues for study were weighed as soon as possible after removal from the body and then dissolved in fuming nitric acid. Their radioactivity was determined on a scale-of-four Geiger Müller counter (1). In addition, the total phosphorus content of the various tissues was determined by the colorimetric method of Fiske and Subbarow (2). Our results are expressed as the specific activity of the tissues per gram mouse (percentage of the original dose of P* per milligram P in 1 gram of tissue per gram mouse).

RESULTS AND DISCUSSION. Our data are treated in three sections: 1, distribution of labeled phosphorus in the mouse; 2, the effect of age on the phosphorus turnover, and 3, the effect of thyroxine on the phosphorus turnover of the mouse.

*Distribution.* The distribution of labeled phosphorus in the mouse is very similar to that found in the rat and other species (3). Expressing our values as per cent dose per gram tissue, we find that tibia shows the highest turnover of

phosphorus followed in descending order by spleen, kidney, liver, heart, blood and brain. A similar order of tissues is reported by Lawrence (4) and associates and by Hodge et al. (unpublished data) on the distribution of phosphorus in the various tissues of the normal mouse. Likewise Cohn and Greenberg (5) in a study of the phosphorus distribution in the young adult rat found that bone shows the largest retention (in per cent dose per gram), followed by liver, stomach and small intestine, heart, kidney, blood and brain.

*Effect of age.* By choosing growing mice we were able to show the changes in the rate of phosphorus turnover in mice with age increase. In table 1 are shown the specific activities of the various tissues of the three age groups under consideration. These values show a statistically significant decrease in the rate

TABLE 1

*Average values for phosphorus turnover in various tissues of albino mice, ages 6, 12 and 24 weeks*

| | PERCENTAGE DOSE RADIOACTIVE PHOSPHORUS PER MILLIGRAM PHOSPHORUS‡ | | | | | | |
|---|---|---|---|---|---|---|---|
| | Blood | Liver | Brain | Spleen | Kidney | Heart | Tibia |
| **Group I. 6 weeks old** | | | | | | | |
| Controls | | 3.10 (10)† | 0.34 (10) | 2.18 (10) | 1.94 (9) | 1.41 (8) | 0.94 (8) |
| Experimentals | | 2.78 (10) | 0.32 (10) | 2.46 (10) | 2.19 (10) | 1.40 (10) | 0.82 (7) |
| Entire group | | 2.94 ±1.17* | 0.33 ±0.11* | 2.32 ±0.58* | 2.07 ±0.78* | 1.40 ±0.47* | 0.88 ±0.26* |
| **Group II. 12 weeks old** | | | | | | | |
| Controls | 1.55 (5) | 2.48 (5) | 0.16 (5) | 1.44 (5) | 1.58 (5) | 1.31 (5) | 0.21 (5) |
| Experimentals | 1.51 (4) | 2.35 (4) | 0.19 (4) | 1.59 (3) | 1.47 (4) | 1.08 (4) | 0.28 (4) |
| Entire group | 1.53 ±0.17* | 2.42 ±0.48* | 0.17 ±0.04* | 1.49 ±0.11* | 1.53 ±0.23* | 1.21 ±0.15* | 0.24 ±0.04* |
| **Group III. 24 weeks old** | | | | | | | |
| Controls | 1.25 (5) | 1.56 (5) | 0.11 (5) | 0.96 (5) | 1.05 (5) | 0.82 (5) | 0.08 (5) |
| Experimentals | 1.09 (9) | 1.46 (10) | 0.12 (10) | 0.86 (9) | 1.14 (10) | 1.08 (10) | 0.13 (10) |
| Entire group | 1.15 ±0.23* | 1.49 ±0.29* | 0.12 ±0.03* | 0.90 ±0.28* | 1.11 ±0.23* | 0.99 ±0.32* | 0.11 ±0.03* |

* Average deviations.
† Figures in parentheses are numbers of mice used.
‡ Expressed per gram mouse in each case.

of phosphorus turnover of each tissue with increasing age.[1] With an age increase from 6 to 24 weeks, there is a decrease in the phosphorus turnover in the liver of 61 per cent, in the kidney of 46 per cent, in the heart of 29 per cent, and in the tibia of 87 per cent (table 1). These observations are in line with those of Weissberger and Harris (7) who found a similar decrease in phosphorus turnover of blood, kidney, genitals and femur with increasing age of the rat. In table 1 data on the phosphorus turnover of the brain show a decrease of 64 per cent in the period observed. This decrease in total phosphorus turnover is similar to that reported by Chaikoff (8) and his collaborators who studied *in vivo* the phospholipid phosphorus turnover of the central nervous system of the rat at various ages and found that the rate of the incorporation of P* into phospholipid fell off

[1] Statistical procedure according to R. A. Fisher (6), p. 128.

markedly with age. These findings were further substantiated by the recent *in vitro* studies by the same author (9), on the manufacture of phospholipid by the brain. In general, the results in regard to the decrease in phosphorus turnover of various tissues with time are consistent with the histochemical findings of Lowry (10) and his associates who observed that the concentration of phosphorus in the cardiac muscle of the rat diminishes with growth.

Figure 1 shows the slope of the decrease in phosphorus turnover with age for each of the tissues studied. The rate of turnover is clearly seen to be a function of the age of the animal. However, there is no direct correlation between the specific activity of a given tissue and the percentage fall in its specific activity with age, i.e., the tissues with the highest specific activity do not necessarily show the greatest decrease with age. Thus, the descending order of the tissues accord-

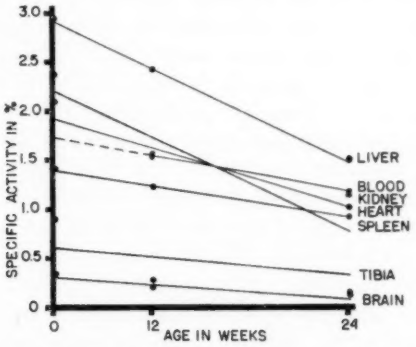


Fig. 1. The decrease in phosphorus turnover with age. At the age of 6 weeks the liver of the mouse has the most active phosphorus turnover; next are the spleen, kidney, blood, and heart in a group, and much lower are the tibia and brain. In the period from 6 to 24 weeks all the tissues show marked decreases in their respective rates of phosphorus turnover. The liver and spleen have the largest decreases in specific activities, the tibia has the greatest percentage decrease (87 per cent), and the brain shows the least change. At 24 weeks the tissues in descending order of activity are liver highest, then blood, kidney, heart and spleen in an intermediate group, and brain and tibia least.

ing to their specific activities at 6 weeks is liver, spleen, kidney, blood, heart, tibia, and brain. However, this order, according to the percentage decrease in the specific activities from 6 to 24 weeks, is tibia, brain, liver, spleen, kidney, blood and heart.

During early growth, large amounts of phospholipid are being laid down in the brain, large amounts of inorganic phosphorus in the bone; phosphorus compounds are not being incorporated into the viscera to as great an extent. Consequently, it is interesting to compare the decrease in phosphorus turnover of these tissues for the two age periods under consideration (6 to 12 wks. and 12 to 24 wks.). This decrease is significantly larger for brain and tibia during the first period than during the second, whereas the specific activities of the viscera decline to about an equal extent for each period.

*Effect of thyroxine.* Since thyroxine is known to have an effect on the basal

metabolic rate, an increase in the phosphorus metabolism was also expected. However, our findings have led to no such general conclusion. The difference between the thyroxine-treated animals and the control values is not statistically significant in any of the tissues investigated, with one exception; there is a valid increase in the phosphorus turnover of the thyroxine-treated older animals (groups II and III, aged 12 and 24 wks. respectively) in the tibia only. There is no such difference, however, in the youngest group of animals (table 1). This may be due to the fact that the phosphorus turnover is extremely high in the young animal and, therefore, the relatively small change induced by the administration of thyroxine may not be demonstrable under our experimental conditions. Belasco and Murlin (11) have reported that in very young rats thyroxine shows less effect on the basal metabolic rate than it does in older animals. In the other tissues (table 1) the apparent slight tendency toward a decrease in turnover following thyroxine administration can be ascribed in all probability to individual variation and experimental error. Manly, Hodge, and Manly (12) have shown that about one-sixth of the bone (so-called labile bone) is in a high metabolic state at all times. From this point of view it is not surprising that the bone phosphorus metabolism is particularly sensitive to thyroxine.

This increase in phosphorus turnover of the tibia following thyroxine treatment has an interesting bearing on the observation of Karnofsky and Cronkite (13) who showed that an excess of thyroid hormone in the rat will produce more rapid appearance and unification of the ossification centers in the bones and a more rapid eruption of the teeth. Furthermore, Silverberg and Silverberg (14) found that in growing mice the administration of thyroxine accelerates and intensifies the age changes of the skeleton. This intensification and acceleration of the skeletal changes which accompany growth is in agreement with our findings of the increased phosphorus turnover of the tibia of the growing thyroxine-treated mouse.

## SUMMARY

In the growing mouse the rate of phosphorus turnover of the tissues studied is a function of the age of the animal.

The decrease in the rate of phosphorus turnover with age is greatest in tibia, followed in descending order by brain, liver, spleen, kidney, blood and heart.

Thyroxine seems to have very little, if any, effect on the phosphorus turnover of the tissues of the mouse, with the exception of the tibia which in the older mice shows an increase in the P* to P ratio upon the administration of thyroxine.

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# LITTER SIZE, GROWTH RATE AND HEAT PRODUCTION OF SUCKLING RATS

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The growth rates of suckling mice (1) and pigs (2) depend on litter size. The smaller the litter the greater the relative milk supply and the more rapid the growth rate. This paper reports data on the influence of litter size on the growth rate of rats during the suckling period (birth to 21 days) and especially on the relative heat productions of the rapidly and slowly growing suckling rats.

Tangl (3), Farkas (4), Bohr and Hasselbalch (5) and others believed that there is a "work of development" (Entwicklungsarbeit), equivalent in birds and insects for the egg-incubation period, when including the maintenance cost, to about a third of the energy content of the unincubated egg. A similar belief was expressed by Terroine and Wurmser (6) for plant growth. Tyler (7) reported that the oxygen consumption (or energy liberation) by the differentiating sea urchin varies with the amount of differentiation.[1] Coleman and Du Bois (8) observed that the basal metabolic rate was 16 per cent above normal in adult typhoid patients when regaining weight during convalescence. It is, moreover, generally known that there is a pubertal metabolic acceleration coinciding with the growth acceleration (9). The pubertal metabolic acceleration may, however, be the result of increased endocrine activity rather than of rapid growth rate as such. This report on the energy increment of growth is confined to the suckling period, thereby avoiding, perhaps, metabolic changes due to endocrine activity.

EXPERIMENTAL. MacDowell and associates (1) working with mice reported maximum growth by reducing the number of young to four at birth, to two at 3 days, and to one at 5 days after birth. Working with Wistar-strain white rats, we observed best growth with reduction of the litter size to four at birth, to three at 2 days, and to two at 4 days. Reducing the litter to one rat sometimes resulted in failure to maintain the normal milk flow.

Oxygen consumption was measured on three average (7, 8, 9) litters, seven two-rat litters and one one-rat litter from birth until weaning at age 21 days.

The measurements were made in a Regnault-Reiset volumetric metabolism apparatus similar in type to the one described by Winchester (10) for fowls. The animals were housed at 27°C. and measured at 30°C.

RESULTS. Table 1 shows that the growth rates of the two groups are about the same until the fifth day when they begin to diverge, the weight curve of the animals in the smaller litters surpassing that of the normal-size litters. The

[1] Collier reported an 85 per cent increase in oxygen consumption at the peak of regeneration of the fasting worm Tubifex tubifex (Jane Graybill Collier: The Relation between Metabolism and Morphogenesis During Regeneration. Doctoral Dissertation, University of Missouri, Columbia, 1942).

instantaneous percentage increases in body weight from the fifth day (when the average weights were the same for both groups) to the 21st day were[2] 158 per cent for the reduced litters and 108 per cent for the normals. The 50 per cent difference presumably reflects differences in milk supply to the two groups, the one whose growth rate was limited by the mother's lactational performance, and the other whose growth was limited by the inherent "growth-impulse" or food-intake capacity.

TABLE 1

*Growth in weight and heat production of suckling rats, members of "normal" litters (7 to 9 per litter) and of reduced litters (1 to 2 per litter)*

| AGE, DAYS | AVERAGE WEIGHT, GRAMS | | AVERAGE CAL. PER RAT PER DAY* | | AVERAGE CAL. PER SQ. METER PER DAY** | |
|---|---|---|---|---|---|---|
| | Normal | Reduced | Normal | Reduced | Normal | Reduced |
| 1 | 5.6 | 5.9 | | | | |
| 2 | 6.2 | 6.1 | 2.12 | 2.15 | 611 | 625 |
| 3 | 7.1 | 6.6 | 2.31 | 2.36 | 611 | 654 |
| 4 | 8.3 | 7.8 | 1.99 | 3.43 | 552 | 855 |
| 5 | 9.7 | 9.7 | 2.70 | 3.13 | 587 | 680 |
| 6 | 10.6 | 11.5 | 3.11 | 4.54 | 639 | 887 |
| 7 | 11.6 | 13.2 | 3.48 | 5.20 | 680 | 930 |
| 8 | 12.8 | 15.2 | 2.98 | 7.43 | 544 | 1,216 |
| 9 | 14.2 | 17.4 | 2.55 | 6.85 | 436 | 1,035 |
| 10 | 15.4 | 18.8 | 3.22 | 7.39 | 520 | 1,059 |
| 11 | 16.3 | 21.8 | 2.97 | 8.16 | 466 | 1,064 |
| 12 | 17.3 | 24.1 | 3.41 | 8.69 | 394 | 1,064 |
| 13 | 18.8 | 24.5 | 4.05 | 7.00 | 580 | 848 |
| 14 | 19.8 | 28.2 | 4.05 | 8.49 | 561 | 941 |
| 15 | 20.8 | 31.2 | 4.23 | 9.34 | 569 | 960 |
| 16 | 21.7 | 33.8 | 4.74 | 9.84 | 620 | 973 |
| 17 | 22.9 | 36.8 | 4.52 | 10.01 | 571 | 939 |
| 18 | 23.5 | 39.4 | 4.73 | 11.31 | 588 | 1,016 |
| 19 | 24.4 | 42.5 | 4.83 | 11.34 | 587 | 972 |
| 20 | 25.3 | 45.4 | 5.29 | 12.22 | 628 | 1,004 |
| 21 | 28.8 | 47.3 | 6.60 | 12.78 | 722 | 1,023 |

* The Calories were computed from oxygen consumption on the assumption that 1 liter of oxygen is equivalent to 4.7 Calories.

** Surface area was computed from the equation surface in square meters = 0.0011 (weight in grams)$^{0.63}$ which is the average of the formulas by Carman and Mitchell (This Journal **76:** 380, 1926), Lee (Idem. **89:** 24, 1929), and Diack (J. Nutrition **3:** 289, 1930).

Table 1 and figure 1 demonstrate a greater oxygen consumption by the more rapidly growing animals regardless of the reference base employed. Thus from figure 1, at body weight 20 grams, the metabolism of the more rapidly growing (smaller litter) animals is about twice as great as of the less rapidly growing (larger litter) animals. Some of this difference in heat production may be

[2] Computed by deducting the natural logarithms of the weights at age 5 days from that at age 21 days.

attributed to differences in specific dynamic action but not to the extent indicated. Some of this difference in heat production may be attributed to difference in "social-temperature regulation" (11) in small and large litters but not to the extent indicated. These data appear to favor the view that there is an organizational energy cost: the more rapid the growth rate, the greater the oxygen consumption or heat-production rate.

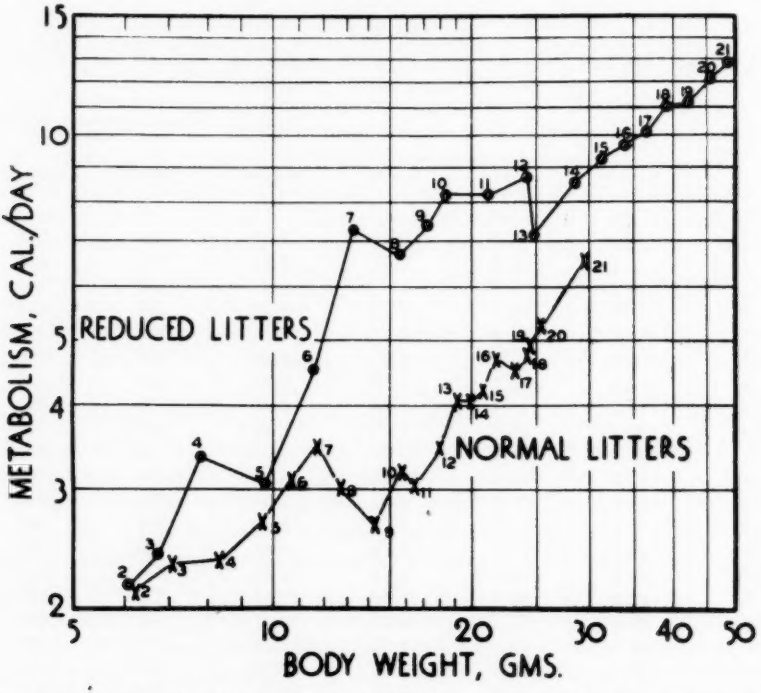


Fig. 1. Average metabolism in terms of Calories per rat per day as function of body weight of the rapidly and normally growing animals. The numerals on the curves represent ages in days counted from birth.

## SUMMARY

This report presents daily averages on increase in weight and in oxygen consumption of suckling rats (up to 21 days), members of average-size litters (7 to 9 rats per litter), and of reduced litters (1 and 2 rats per litter). Beginning with the fifth day after birth, the average growth rate of the rats in the small litters was greater than that of the rats in the normal litters. The rapidly growing rats exhibited a higher heat production, regardless of the reference base employed, than did the normals. This is interpreted as evidence for the view that there is an organizational energy expense to growth and morphogenesis aside from the energy stored or other types of energy resident in the structure, as for example the energy of structural orientation postulated by Needham (12).

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# THE EFFECT OF A PROTEIN-DEFICIENT DIET ON THE SERUM PHOSPHATASE AND HEPATIC DYE CLEARANCE OF DOGS

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Diets deficient in protein have been shown to produce changes in the function, structure, and possibly the permeability of the liver cell (1, 2, 3, 4, 5, 6). There is considerable evidence to indicate that an elevation of the serum phosphatase may be a sensitive indicator of certain alterations in liver function (7, 8, 9, 10, 11, 12).

The present study was undertaken to determine whether a protein deficiency would produce an elevation of the serum phosphatase of dogs. This question was answered in the affirmative. Next, a hepatic dye clearance test and the serum phosphatase were studied simultaneously in protein-deficient dogs to ascertain to what extent these two tests behaved similarly. It was found that the striking increase in the serum phosphatase produced by a protein deficiency paralleled closely the reduction in hepatic dye clearance produced by this deficiency. The changes observed in these tests are reversible and may be brought back to the normal range by adding one of the several so-called complete dietary proteins to the diet.

METHODS. Adult mongrel dogs were used in all the experiments. The general procedure was to maintain the animals for about two weeks on an adequate diet during which time control values for serum inorganic phosphorus, serum phosphatase and hepatic dye clearance were obtained. Any animal showing abnormalities in appetite, activity or blood values was discarded. Some of the animals were then placed on the deficient diet, others on the control diet, and all were bled regularly at two week intervals for phosphorus and phosphatase determinations. In the third group of dogs hepatic dye clearance was also determined at two week intervals. When the animals became undernourished due to the gradual development of an anorexia they were sacrificed and immediately autopsied.

The protein-deficient diet which was fed at a level of forty calories per pound consisted of sucrose 55 per cent, lard 33 per cent, yeast (Anheuser-Busch Strain K) 5 per cent, Wesson's salt mixture 2 per cent, and ground cellophane 5 per cent. To each one hundred grams of diet were added 200 units of vitamin D and 1400 units of vitamin A in percomorph oil. The control diet was similar except that 25 per cent of the sucrose was replaced by protein in the form of either casein (both Labco vitamin-free and a commercial grade were used) or cooked egg white. The control dogs remained in a healthy state until the time they were sacrificed, which was between six and twelve months after the beginning of the experiment.

All blood determinations were made after at least twelve hours of fasting. Serum inorganic phophorus was determined by Bodansky's modification (13) of the Kutner-Lichtenstein method (14), using the Klett-Summerson photoelectric colorimeter. Serum phosphatase activity was determined by Bodansky's method (15). The method of Best, Channon and Ridout (1) was used to determine the total lipid content of the fresh liver. Hepatic dye clearance was measured by the Rose Bengal dye clearance test of Stowe, Delprat and Weeks (16). In using this test it was found convenient to use graded doses (0.14 cc./lb. of a 2 per cent solution in saline) and also to precipitate the proteins with eight parts of acetone to one part of serum. This gave filtrates which remained clear for long periods of time and could be conveniently read in the photolometer using a filter giving maximum transmission at 5400 Å. In using this test the time intervals were carefully controlled with a stop watch and it was found that the test could be repeated on the same animal with an accuracy greater than 90 per cent.

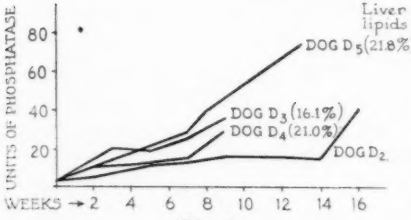


Fig. 1

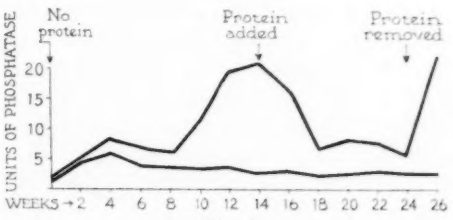


Fig. 2

Fig. 2. Upper line: average serum phosphatase of 5 dogs maintained on a protein-deficient diet with supplements as indicated.

Lower line: average serum phosphatase of 2 dogs maintained on protein-control diet. One per cent choline was included in the diet of this group from the 4th to the 6th weeks.

Results. The gradual increase in the serum phosphatase which resulted from a protein-deficient diet is shown in figure 1. These four dogs are typical of the twenty-four protein-deficient animals which manifested this increase during 34 depletion periods. The control dogs receiving protein showed no significant increase in serum phosphatase. Normal adult dogs on an adequate diet usually have a serum phosphatase activity between one and four Bodansky units. The time required for an animal being depleted to show a rise above this range was found to vary from two weeks in some animals to eight weeks in others. The level to which the phosphatase activity rose also varied and seemed to depend to some extent upon the age of the animal, younger animals exhibiting the higher values. The increases observed in adult dogs have varied from ten to seventy-four Bodansky units per hundred cubic centimeters of serum. By the time the elevation of the serum phosphatase had reached what appeared to be a maximum the animals began to refuse a portion of their food, had lost 10 to 15 per cent of their body weight and in most cases had developed trophic ulcers on their extremities. Only two of the twenty-four treated dogs showed evidence of an

edema at the height of the first depletion. Some of the animals were sacrificed then and the fat content of the fresh liver was determined. The livers of $D_3$, $D_4$ and $D_5$ contained 16.1, 21.0 and 21.8 per cent fat respectively. A yellowish-brown pigmentation of the pancreas and small intestine was a conspicuous autopsy finding in many of the dogs. Chronic duodenal ulcers were found in four of the animals which had been depleted more than once. The liver cells were largely replaced by droplets of fat. The infiltration of fat was greatest about the periphery of the functional lobule of the liver. There was no significant increase in fibrous tissue in the livers of any of these animals.

In figure 2 is shown the result of returning protein to the diet of the depleted animal. Either casein or cooked egg white fed at a level of 25 per cent of the diet brought about a decrease in the serum phosphatase. The incomplete proteins, gelatin and zein were fed to a few animals, but because they would not

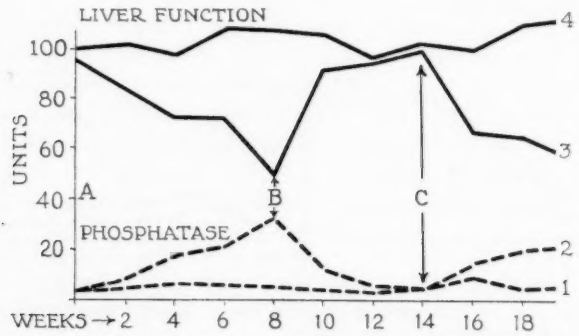


Fig. 3. *1*, serum phosphatase of 2 control dogs. *2*, average serum phosphatase of 3 treated dogs. *3*, average liver function of 3 treated dogs. *4*, liver function of 2 control dogs.

*A*, treated dogs started deficient diet. *B*, protein added to diet of treated dogs. *C*, protein removed from diet of treated dogs.

consume the diet in amounts adequate for maintenance the results are difficult to interpret and will not be considered here. When casein or egg white were added to the diet the phosphatase returned to essentially normal values in two to four weeks. However, changes in the general behavior of the animals were seen much sooner. A dog that had become emaciated and inactive during a protein-deficient regime and had reduced his caloric intake to as little as 50 to 100 calories per day, almost immediately began eating adequate amounts of food and became more animated. In two to three weeks the ten to fifteen per cent weight loss had been regained. After four weeks on the experiment this group received 1 per cent choline chloride in the diet for two weeks. This probably accounts for the longer time required to complete the depletion of the deficient animals as compared to those shown in figures 1 and 3.

In figure 3 the relation which exists between the increase in serum phosphatase and the decrease in hepatic dye clearance is shown. The upper solid line and

lower dotted line represent the per cent of normal dye clearance and serum phosphatase respectively of two control dogs. The middle two lines represent the hepatic dye clearance and phosphatase of three treated dogs. Eleven other dogs in fourteen experiments have given almost identical results. In practically every case where the phosphatase increased the hepatic dye clearance decreased and in most cases where the enzyme activity decreased, the dye clearance increased. However, the exceptions to this last generalization require an explanation.

Two of the animals which had been depleted to the point where their phosphatase had increased and their hepatic dye clearance had decreased were fed beef heart fractions as a supplement to the basal protein-deficient diet. The fractions used were kindly supplied by Dr. David Klein of the Wilson Laboratories and consisted of the residue from repeated hot water extractions of fresh beef hearts and the concentrated water extract. The residue or extractives derived from 2 grams of dry whole beef heart protein was fed for each pound of body weight. When fed the residue for six weeks a decrease from 9.1 units to 4.5 units of phosphatase and an increase in hepatic dye clearance from 72 per cent of normal to 93 per cent occurred in dog 1-E. Dog 5-E in six weeks exhibited a decrease in phosphatase from 47.2 units to 11.1 units and an increase in hepatic dye clearance from 35 per cent of normal to 63 per cent. Both these responses were positive ones, but the magnitude of the change was roughly only about two-thirds of the response a similar amount of casein or egg white would have brought about. When the concentrated water extract was fed in equivalent amounts there occurred in dog 1-E after two weeks a slight rise in phosphatase and a decrease in hepatic dye clearance from 80 per cent to 73 per cent. Dog 5-E showed a rise in phosphatase from 11.1 units to 21.3 units in 2 weeks and a decrease in hepatic dye clearance from 63 per cent of normal to 35 per cent. At this time the animal developed ascites and was estimated to have about two liters of fluid in his peritoneal cavity. One other animal was fed the extract but died suddenly before a second set of determinations could be made.

Discussion. With the exception of studies in which rachitogenic diets were fed, only a few experiments have been reported in which the serum phosphatase activity of dogs was shown to be influenced by the diet. Bodansky (17, 18) reported increases in the serum phosphatase of dogs after feeding fasted animals large amounts of carbohydrate. He also demonstrated that nursing puppies have a higher serum phosphatase activity than fasting puppies. These values were obtained on non-fasting blood and are believed by Bodansky to be due to the increased carbohydrate metabolism. Since the results being reported here were obtained on fasting serum they cannot be compared with the transitory rises observed by Bodansky. Freeman and Farmer (19) found that the fasting serum phosphatase values of dogs maintained on high protein diets (beef hearts) were consistently lower than values obtained on high carbohydrate (bread and meal) diets. The nature of the changes they observed are probably of a similar origin to those reported here.

Numerous studies (7, 8, 9, 10, 11, 12) have been made of the increase in serum phosphatase which occurs in certain types of liver damage other than the fatty infiltration due to a protein deficiency. None of these studies has, however, furnished indisputable evidence in regard to the origin of the serum phosphatase. The greater part of the evidence points to the liver as an important source, and seems to indicate that a liver slightly damaged or irritated by various factors produces increased amounts of the enzyme, but an extensively damaged liver cannot do this. Freeman, Chen and Ivy (12), in order to show whether or not the increase in serum phosphatase which results from a partial obstruction of the liver is due merely to the loss of excretory function, obstructed a lobe of the liver in several dogs and obtained a prolonged increase in the phosphatase activity. In a second group of dogs they removed an amount of liver tissue equal to that which had been obstructed in the first series and did not find a comparable increase in the enzyme in the serum. Schiffmann and Winkelman (20) have shown that the serum phosphatase elevation following obstruction of the common bile duct is greater in normal animals than in those previously poisoned with arsenic trioxide. Since this compound injures the liver, it may be inferred that the difference in serum phosphatase resulted from injury to the liver.

The experiments reported here provide no basis for determining the site of origin of the serum phosphatase. The changes occurring concomitantly in the liver, i.e., an increase in the lipid content, a decrease in the cell cytoplasm, and a decrease in the protein content (21) suggest that the increased phosphatase activity may be due to the loss of excretory function, alterations in cell permeability, or both. The decreased ability of the liver to remove intravenously injected Rose Bengal is a sensitive indicator of the excretory changes which are occurring. The possibility that the increased enzyme activity is due to a change in the concentration in the blood of an activator or inhibitor substance has not been ruled out.

The effect of the proteins, casein and egg white, in bringing about a decrease in the serum phosphatase and an increase in the hepatic dye clearance may be due to their lipotropic properties (1, 2, 3, 4, 5, 6). The lipotropic effect of proteins is now believed to be dependent upon the ratio of methionine to cystine in the dietary protein (22). Du Vigneaud et al. (23) have postulated that the lipotropic effect of methionine is due to the ability of this amino acid to furnish methyl groups for the synthesis of choline within the body, and that it is the choline which directly or indirectly brings about the reduction of liver fat.

It is well established that chemical, mechanical, and bacterial agents which injure the liver are capable of causing an elevation of the serum phosphatase. In the interpretation of abnormally high serum phosphatase values of unknown etiology one should also consider certain nutritional deficiencies which affect the liver, particularly those which may result in a protein deficiency.

## SUMMARY

In 34 experiments on 24 dogs it has been demonstrated that the removal of protein from the diet causes an increase in the serum phosphatase and a decrease

in the hepatic clearance of Rose Bengal. These changes occur relatively early in the protein-deficient dog. The addition of protein in the form of either casein or cooked egg white will reverse these findings.

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